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SOIL SCIENCE

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Contents

The Influence of the Decomposition of Organic Matter on the Oxidation-Reduction Potential of Soils. WILLIAM BURROWS AND THEONE C. CORDON 1
The Behavior of Lignin and Humic Acid Preparations Toward a Bromination Treatment. IRVIN C. FEUSTEL AND HORACE G. BYERS 11
Reclamation of Alkali Soils by Electrodialysis. AMAR NATH PURI AND BALMOKAND ANAND 23
The Effect of Calcium Ions and Reaction upon the Solubility of Phosphorus. E. J. BENNE, A. T. PERKINS AND H. H. KING 29
Estimation of Available Phosphates in Soils by CO_2 Extraction. AMAR NATH PURI AND A. G. ASGHAR 39
Estimating Exchangeable Calcium and other Cations in Soils. AMAR NATH PURI 47
Root Nodule Bacteria of Some Tropical Leguminous Plants: I. Cross-Inoculation Studies with *Vigna Sinensis L.* O. N. ALLEN AND ETHEL K. ALLEN 61

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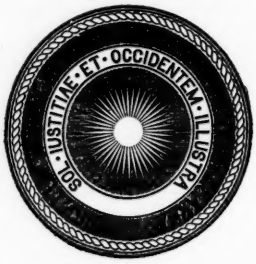

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THE INFLUENCE OF THE DECOMPOSITION OF ORGANIC MATTER ON THE OXIDATION-REDUCTION POTENTIAL OF SOILS[1]

WILLIAM BURROWS AND THEONE C. CORDON[2]

New Jersey Agricultural Experiment Station

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The study of potentials developed in soils, begun by Gillespie in 1920 (6), has been continued sporadically through the following years. Considerable progress has been made in this connection, as brought out by the work of Remezow (13), Willis (14), Heintze (9, 10), Brown (3), Bradfield, Batjer, and Oskamp (2), and Peech and Batjer (11). The E_h-pH relationship, the relation of potential to the formation of ferrous compounds, and the relation of potential to fertility in orchard soils have been carefully studied. From a practical soil point of view, the last may be particularly significant. As far as the authors are aware, however, no effort has been made to study the development of oxidation-reduction potential in relation to the decomposition of various types of organic matter in standing soil. In view of the theoretical and possible practical importance of this problem in soil investigation the present experimental work has been undertaken in an effort to throw some light on the effect of such decomposition and attendant microbial activity on the reducing intensity of the soil.

The observations recorded here are corrected to pH 7.0. The intimate relation between pH and E_h makes some degree of correction esssential when pH varies as widely as in the present experiments. Willis (14), making measurements on soils suspended in water, found a pH-E_h slope of 0.060 volt per unit change in pH. This is in good agreement with the theoretical value 0.059 for the quinone-hydroquinone system and certain other systems in which an exchange of two electrons is involved. Peech and Batjer (11), however, found a somewhat steeper slope when measurements were made on soil suspended in 0.1 *N* H_2SO_4 and used a correction factor of 0.080 volts per unit change in pH. They discuss the degrees of slope that accompany various oxidation-reduction systems in which varying numbers of electrons are concerned as well as the conditions of their own experiments, such as the evolution of CO_2. In the present experiments the smaller factor 0.060 is used in

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[2] The authors wish to acknowledge their indebtedness to Dr. Selman A. Waksman, who suggested this investigation and whose critical advice throughout the work has proved invaluable.

order to minimize the differences in potential level attained in the presence of organic substances of a protein or carbohydrate nature. At present any correction factor can be no more than a guess. This uncertainty is particularly true in the experiments reported here in which oxidation of substrates differing widely in their nature is taking place—very possibly different systems with different E_h-pH relations are involved (1).

The practice of suspending soil in water or dilute acid solutions prior to measuring oxidation-reduction potential is open to some criticism. It is quite possible that dissolved oxygen in such liquids will affect relatively poorly poised systems to a considerable degree. In the experiments under consideration here wide and unpredictable fluctuations have been observed, not only in comparing values obtained from direct measurements on soil with those obtained on water suspensions of the same soil (prepared for pH determinations), but also among duplicate samples of soil subjected to such treatment. We have, therefore, endeavored to make potential measurements on soil samples disturbed as little as possible. In this way, fairly good agreement between duplicate pots has been obtained. It may be added in this connection that sand media give much closer agreement between duplicate electrodes and duplicate pots than do soil mixtures and are, therefore, preferable to the latter.

EXPERIMENTAL

The electrodes were bright platinum wire, 1.5 inches long. These were inserted directly into the soil sample. An attempt was made to use bright platinum plates made rigid by means of a sealed on glass support, but these were unsatisfactory. Duplicate electrodes were used throughout. The salt bridge, which was also inserted into the soil sample, was a saturated solution of KCl so arranged that the entire bridge could be flushed out with fresh salt solution after each measurement. As will be pointed out later, it is of the greatest importance that the bridge be thoroughly cleaned between samples, an operation that is not possible with agar bridges. The remainder of the apparatus was of the usual type [that used here has been described (4)].

The soil used in these experiments was a sassafras sandy loam. Preliminary tests indicated that potential measurements could be made on such a soil when it contained 20 per cent or more moisture. The optimum moisture content for the activities of aerobic organisms, namely, 20 to 24 per cent, was, therefore, used throughout the experiments. The added material, casein, starch, etc., was mixed with the dry soil, and the mixture was placed in pots. Distilled water was added to bring the moisture content to the desired level. Two to three hours was allowed to elapse between the addition of water and the first removal of samples. Presumably during this time the moisture became more or less uniformly distributed, and it was assumed that no activity capable of bringing about changes in oxidation-reduction potential had taken place, the time of removal of the first sample being recorded as 0 hours. Every experiment was run with duplicate pots each of which contained

1000 to 1500 gm. of soil, on a dry basis. The pots were covered with glass plates and kept at a constant temperature of 28°C.

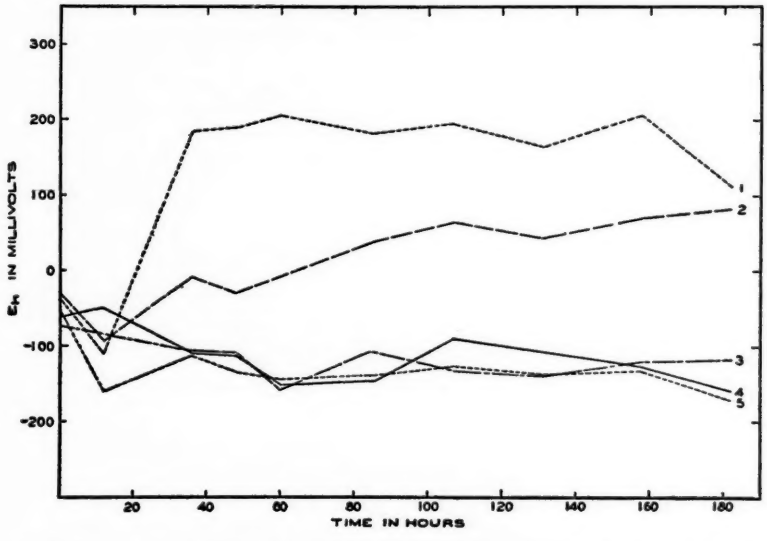

FIG. 1. EFFECT OF THE ADDITION OF NITROGENOUS AND CARBOHYDRATE MATERIALS ON THE POTENTIALS DEVELOPED IN SOIL

1, casein; 2, alanine; 3, dextrose; 4, control; 5, starch

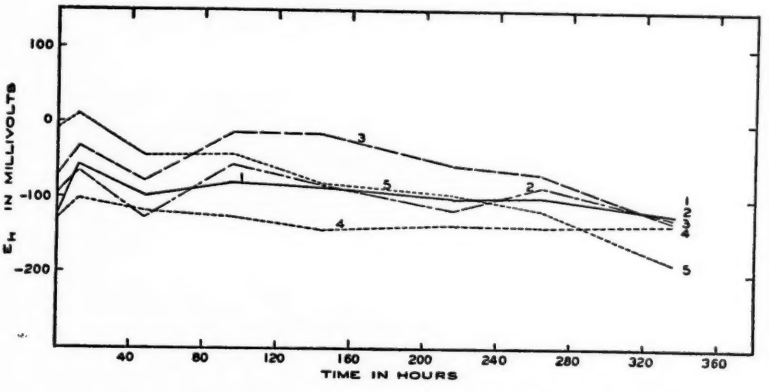

FIG. 2. EFFECT OF THE ADDITION OF COMPLEX ORGANIC MATTER ON THE POTENTIAL OF SOIL

1, stable manure; 2, rye straw; 3, fresh cut grass; 4, control; 5, rye straw + $(NH_4)_2HPO_4$

Samples of approximately 10 gm. were taken from time to time from the pots with a spatula or in a column removed with a large cork borer (the

method used appeared to make little difference). The samples were placed in 100-cc. beakers. When the sample was removed with a spatula, some packing in the beaker was necessary, but when the cork borer was used, the

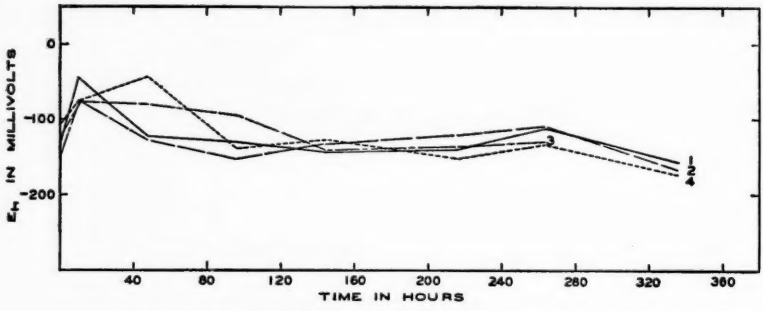

FIG. 3. EFFECT OF MOISTURE ON THE POTENTIAL OF STANDING SOIL
1, 24 per cent moisture; 2, 36 per cent moisture; 3, 18 per cent moisture; 4, 30 per cent moisture

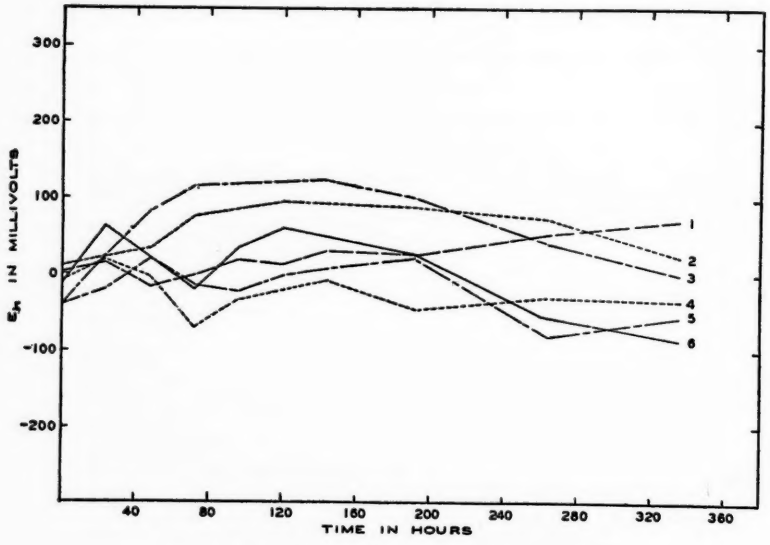

FIG. 4. SAND MEDIUM WITH A CARBOHYDRATE BASE
1, *Actinomyces californicus;* 2, *A. bobili;* 3, *Trichoderma* sp.; 4, *Bacterium fluorescens;* 5, *B. cereus;* 6, *Mucor* sp.

column of soil was expelled into the beaker without unnecessary disturbance, and, therefore, no packing was needed. After the potential measurements had been made, the soil samples were suspended in 50 cc. of distilled water,

the suspensions were allowed to stand for 2 hours, and the pH was determined by means of the glass electrode.

Part I. A quantity of air-dried soil was divided into 10 portions of 1500 gm. each. Two portions received 0.5 per cent casein; two portions, 0.5 per cent alanine; two, 0.5 per cent starch and 0.1 per cent $(NH_4)_2HPO_4$; two, 0.5 per cent dextrose and 0.1 per cent $(NH_4)_2HPO_4$; and two portions were left as controls. After thorough mixing, the soil and added ingredients were transferred to individual pots, and sufficient water was added to bring the moisture content to 20 per cent. E_h and pH measurements were made on samples removed from time to time. The results, corrected to pH 7.0, appear in figure 1.

Part II. Ten portions of 1000 gm. each of air-dried soil were used. Of these, two were unaltered; two contained 1 per cent by weight of rye straw; two, 1 per cent rye straw and

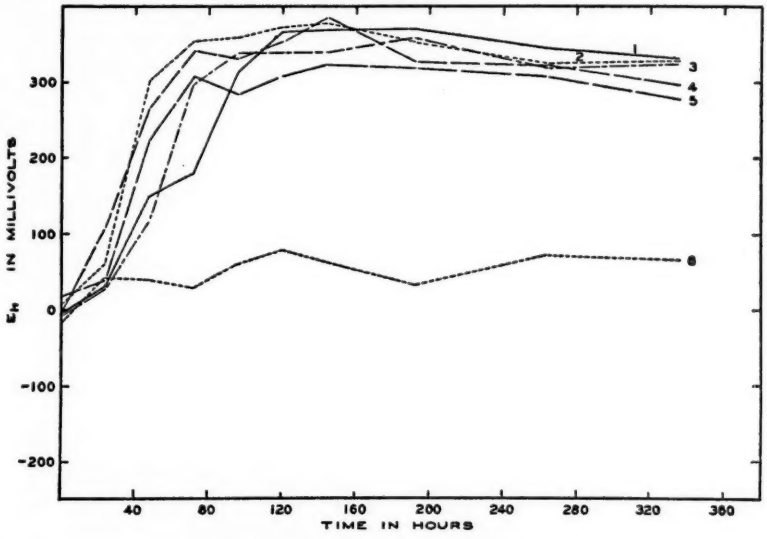

FIG. 5. SAND MEDIUM WITH A PROTEIN BASE

1, *Actinomyces californicus;* 2, *Bacterium cereus;* 3, *Actinomyces bobili;* 4, *Mucor* sp.; 5, *Trichoderma* sp.; (6), *Bacterium fluorescens*

0.1 per cent $(NH_4)_2HPO_4$; two, 4 per cent fresh cut grass; and two, 4 per cent fresh stable manure. The soils were distributed in individual pots, and water was added to bring the moisture content to 24 per cent. Potential changes in these mixtures over the period of observation are shown in figure 2.

Part III. Ten 1000-gm. portions of soil were distributed in individual pots, and water was added to bring the moisture content of pairs to 12 per cent, 18 per cent, 24 per cent, 30 per cent, and 36 per cent respectively. Changes in potential under these varying conditions of moisture are shown in figure 3.

Part IV. Two sand media were prepared; one with a protein base, 1 per cent casein, and 0.01 per cent K_2HPO_4, and the other with a carbohydrate base, 1 per cent dextrose, together with 0.02 per cent $NaNO_3$ and 0.01 per cent K_2HPO_4. Two hundred gram quantities of the sterile media in wide-mouthed Erlenmeyer flasks were inoculated with pure cultures

TABLE 1

Changes in potential as a result of different soil treatment

E_h calculated to pH 7.0, using the factor 0.060 volt per unit pH

Soil Media—Unsterilized

	POT* NUMBER		TIME IN HOURS									
			0	12	36	48	60	85	107	131	158	182
20 per cent moisture	1	Control	−65	−53	−112	−113	−155	−149	−93	−162	−129	−160
	2	Casein	−42	−113	+181	+187	+204	+180	+192	+163	+204	+109
	3	Alanine	−33	−94	−1	−31	−13	+38	+64	+43	+71	+83
	4	Starch + $(NH_4)_2HPO_4$	−53	−161	−111	−134	−144	−138	−126	−136	−131	−152
	5	Dextrose + $(NH_4)_2HPO_4$	−74	−85	−106	−109	−159	−107	−131	−136	−120	−116
			0	12	48	96	144	216	264	336	552	
24 per cent moisture	6	Rye straw	−94	−65	−126	−54	−81	−113	−82	−124	−88	
	7	Rye straw + $(NH_4)_2HPO_4$	−8	+13	−43	−41	−81	−93	−114	−185	−136	
	8	Fresh cut grass	−71	−33	−79	−13	−14	−56	−66	−130	−148	
	9	Fresh stable manure	−124	−57	−98	−79	−84	−99	−96	−121	−97	
	10	Control	−130	−102	−119	−126	−143	−136	−138	−132	−108	
	11	12 per cent moisture	−140	−142								
	12	18 per cent moisture	−151	−76	−80	−95	−141	−137	−130			
	13	24 per cent moisture	−133	−45	−123	−131	−142	−140	−111	−158		
	14	30 per cent moisture	−125	−74	−43	−139	−127	−152	−134	−174	−117	
	15	36 per cent moisture	−106	−75	−125	−151	−131	−147	−108	−168	−111	

Sand Media—Sterilized

			0	24	48	72	96	120	144	192	264	336
20 per cent moisture	16	Glucose—*Bacterium cereus*	−40	−21	+19	−18	−23	−2	+7	+22	−81	−55
	17	Glucose—*B. fluorescens*	−10	+20	−6	−71	−35	−10	−10	−46	−30	−35
	18	Glucose—*Trichoderma*	−41	+24	+84	+116	+118	+119	+124	+101	+41	0
	19	Glucose—*Mucor*	−15	+62	+22	−21	+34	+61	+46	+24	−54	−85
	20	Glucose—*Actinomyces californicus*	−1	+14	−18	−1	+18	+12	+30	+25	+54	+72
	21	Glucose—*A. bobili*	+8	+20	+32	+76	+97	+94	+88	+99	+74	+23
	22	Casein—*Bacterium cereus*	+6	+60	+301	+354	+359	+373	+378	+353	+326	+328
	23	Casein—*B. fluorescens*	−16	+42	+40	+29	+60	+79	+64	+33	+73	+66
	24	Casein—*Trichoderma*	+16	+37	+223	+307	+283	+308	+323	+319	+308	+279
	25	Casein—*Mucor*	−3	+106	+267	+342	+331	+354	+386	+327	+324	+298
	26	Casein—*Actinomyces californicus*	−7	+30	+148	+179	+313	+366	+369	+370	+346	+332
	27	Casein—*A. bobili*	−10	+29	+115	+297	+339	+341	+339	+333	+321	+328

* Each pot number represents a pair of pots, and, therefore, each value is the average of duplicate experiments (four actual measurements) rounded to the nearest millivolt.

of soil microörganisms. At the end of the manipulation each medium contained 20 per cent moisture. The experiments were run in duplicate, i.e., each microörganism was grown in two flasks of each medium, or four flasks in all. The following microörganisms were used: *Bacterium cereus*, *B. fluorescens*, *Trichoderma* sp., *Mucor* sp., *Actinomyces californicus*, *A. bobili*. The time-potential curves of these experiments, corrected to pH 7.0, are shown in figures 4 and 5.

A summary of the results of all the foregoing experiments is given in table 1.

DISCUSSION

The error inherent in EMF measurements on poorly poised material, such as in the present experiments, is large. It is probably not so great as some of the data, as in figures 2 and 4, would seem to indicate, for here very possibly microbial species differences exert their influence. Figure 3 is a better illustration of variation due to experimental error. Species differences in oxidation-reduction potential, first pointed out by Burrows and Jordan (4) and later confirmed by Gillespie and Rettger (8) are a very real factor in the determination of the degree of reducing intensity that occurs. It must be borne in mind that in a medium such as unsterilized soil, which contains a great variety of microörganisms, addition of various kinds of decomposable organic matter will result in the predominance of a certain type of population. Thus, observed differences in potential resulting from the addition of different kinds of organic matter may, in some cases, be the result of the activity of a different microbial flora.

Although the soil media used in the present experiments are exceedingly complex, both biologically and biochemically, the results of the first portion of the work definitely indicate that a very marked difference in potential occurs when casein or alanine and dextrose or starch are undergoing decomposition in unsterilized soil. This difference is considerably greater than the experimental error and is not the result of differences in pH, since the introduction of any correction factor for the latter tends to accentuate the wideness of separation of potential levels. That this difference is real and not the result of different types of microbial flora is apparent from the results of the experiments of Part IV. When pure cultures of soil microörganisms were grown in sterile sand media the differences in potential level in the presence of carbohydrate and nitrogenous substrates became more marked than in soil media. In the former case the same species were tested on both nitrogenous and carbohydrate substrates and resulted in striking evidence of the effect of the type of nutrient on the potential produced in cultures of microorganisms. The association of a strongly positive potential level with the decomposition of casein is shown in figure 5 in which *Bact. fluorescens* alone remains at a level comparable to that attained in the carbohydrate medium. Since this organism does not decompose casein, its remaining at a lower level seems particularly indicative of an intimate relation between the protein decomposition and positive potential level.

27	Casein—*A. bobili*	−10	+29	+115	+297	+339	+341	+339	+333	+321	+328

* Each pot number represents a pair of pots, and, therefore, each value is the average of duplicate experiments (four actual measurements) rounded to the nearest millivolt.

This association of positive potential with protein decomposition does not appear to agree with the probable activity of obligate anaerobes, which is known to be favored by the conditions of the present experiments, i.e., a rather vigorous oxidation which undoubtedly results in a rapid depletion of the oxygen supply, a supply of readily available nutrient material, and an alkaline reaction. Dack and Burrows (5) have shown, however, that some of the non-sporulating obligate anaerobes do not produce the extremely negative potentials (approaching hydrogen over-voltage) that have been found in cultures of some sporulating obligate anaerobes (12). These non-sporulating forms did not differ, in respect to potential, from the usual aerobic types. It is quite possible, therefore, that obligate anaerobic bacteria may be active in some of the present experiments in spite of the positive potential levels found to exist.

In this connection it is appropriate to point out that the statement of Heintze (9) that the negative drift in potential in standing soil is an indicator of organic matter content should be modified, since it is apparent that a negative or positive drift depends on the *nature* of the organic matter.

The results shown in figure 2 are, perhaps, deserving of comment in that qualitative differences in the added decomposable material do not result in marked differences in potential level. In these cases, of course, the results are not strictly comparable, for new bacteria were added in the form of contamination, such as in stable manure, and, therefore, the microbiological population may have differed considerably from pot to pot. It is possible that real differences do exist, but the experimental work presented here is not adequate to demonstrate them. In order to determine relatively small differences such as these, if they exist, it is necessary to resort to some statistical method such as that used by Burrows and Jordan (4); otherwise, it can hardly be said that "significant" differences are present. The complex nature of the added material and of the soil, together with present lack of information, precludes further speculation in this connection.

The negligible effect of moisture content on potentials developed in soil on standing may be noted. As has already been pointed out, apparently there is no rigid relation between the exclusion of oxygen and the potential developed. This is in agreement with the conclusion reached by Heintze (9), who found that potential was no criterion of water-logging.

In the present experiments we have not had the difficulty referred to by Peech and Batjer (11) of potential drift and the necessity of aging electrodes in solutions of proper potential before measurements could be made. This problem did arise in the measurement of potentials of muds from sea bottoms,[3] but it was found at the time that this was due to inadequate cleaning of bridges between samples. Agar bridges could not be thoroughly cleaned, but when saturated KCl solution bridges were used and flushed with con-

[3] Unpublished experiments.

siderable quantities of fresh solution, in addition to careful cleaning on the outside, between samples, this difficulty was not experienced, and a series of samples, varying considerably in their potentials, could be read in any order without affecting the final result. This technique was, therefore, continued in soil experiments and was found to be entirely satisfactory.

SUMMARY

The experimental evidence presented here indicates that the type of decomposable organic matter present in the soil is a highly important factor in the determination of the degree of reducing intensity that will prevail. Decomposition of casein was found to result in strongly positive potential levels, whereas decomposition of carbohydrate produced more negative, but not extremely negative, potentials which do not appear to differ greatly from those produced in standing soil to which no organic matter was added. The effect of moisture content on potentials developed in standing soils was found to be negligible.

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THE BEHAVIOR OF LIGNIN AND HUMIC ACID PREPARATIONS TOWARD A BROMINATION TREATMENT

IRVIN C. FEUSTEL AND HORACE G. BYERS

Bureau of Chemistry and Soils, U. S. Department of Agriculture

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Various chemical characteristics and physical properties of the so-called "humic acids" have been investigated for many years. The progress of chemistry, however, in the study of these organic complexes resulting from the decomposition of plant material has been very slow. The reasons for this are chiefly that humic matter possesses a high molecular weight, is for the most part insoluble except in alkali, is extremely resistant to breakdown into simpler derivatives, and finally, is of variable composition depending upon the source and method of preparation.

The terms "humus" and "humic acid" are vague and indefinite because of indefinite chemical characterization but must be considered in either case as referring to a group of complex substances which compose a relatively large proportion of peat or of soil organic matter. In this paper the term "humic acids" or "humic acid fraction" is used to refer to the fraction of the organic matter dissolved by dilute alkali solution under pressure and precipitated by mineral acid. The lignin or lignin degradation products present in peat or soil organic matter are contained largely, if not completely, in this particular fraction.

The object of the present investigation was to demonstrate fundamental differences or similarities in the character of derivatives of several humic acid preparations when compared with one another and when compared with a lignin compound. These materials were prepared in the same manner and were subjected to a bromination treatment which presumably by simultaneous oxidation, substitution, and addition reactions effected very drastic alterations. The resulting products were largely ether- and alcohol-soluble; this property among others permitted an examination not possible with the original substances. Crystalline derivatives were also noted as having been formed during the bromination reaction, and it was hoped that the identification of these by-products would yield further information as to the nature of the organic materials and particularly the relationship of the humic acids to lignin.

REVIEW OF LITERATURE

Previous experiments (9) on the bromination of humic acids suspended in carbon tetrachloride indicated that practically no addition occurred but that from 22.80 to 51.53 per cent

of bromine was substituted. Similarly, the principal reaction found to take place with lignin was that of substitution, with a simultaneous splitting off of methoxyl groups (5). Friedrich and Pelikan (7) distinguished differences in the character of the methoxyl groups of lignin since only a portion could be removed by bromine. When wood is treated with bromine in the presence of water, according to Fuchs (8), the lignin is gradually destroyed whereas cellulose is not attacked. Coal may absorb 30 to 36 per cent of bromine by the action of aqueous bromine (8).

Other bromination studies (6) with lignin report the formation of quinone-like substances. Klein (10, p. 326) states that in the treatment of humic acids with potassium chlorate and hydrochloric acid small amounts of chloranil (tetrachlorquinone) are found. The formation of crystalline compounds of unknown constitution resulting from the bromination of different varieties of peat has been demonstrated (4).

On the basis of the solubility of peat in a sulfurous acid solution after removal of ether-soluble substances, Oden and Lindberg (12) concluded that a low lignin content paralleled a high degree of humification. Similar results (17) were obtained by the use of a chlorine dioxide solution to distinguish lignin from humic matter, the latter being soluble only in alkalies.

Differences in the chemical behavior of soil organic matter from different soils have been shown by Shorey (16) by means of a variety of reactions. The extensive work on humus in general has very recently been reviewed in a complete manner by Waksman (18).

MATERIALS AND METHODS

Two samples of peat, one of lignin, and one of soil organic matter were used in the bromination experiments. The peats included a sawgrass variety obtained from Zellwood, Florida, and a sphagnum moss peat from Cottage Lake near Seattle, Washington. The former was partially decomposed and moderately acid, whereas the latter was extremely acid and but very slightly decomposed. The sample of lignin was kindly furnished by Dr. Max Phillips, of this bureau, who prepared the sample from corn cobs by the alkali method (13). The source of soil organic matter was a sample of Marshall silt loam obtained from the erosion experiment station near Clarinda, Iowa. The physical and chemical characteristics of this soil have previously been described in detail (11).

Each material was ground to pass a 1-mm. mesh sieve and used in an air-dry condition for all subsequent experiments.

The humic acids were prepared by treatment of 200 gm. of peat or 2,000 gm. of soil with 2 liters of a 2 per cent solution of sodium hydroxide at 130°C., or approximately 25 pounds gauge pressure. An autoclave of 1 gallon capacity was used, and the duration of the treatment was 6 hours. The reaction mixture was filtered through a Pasteur-Chamberland filter after cooling and removal from the autoclave. The filtrate was treated with an excess of hydrochloric acid, boiled for one-half hour, and again filtered. The acid precipitate was washed several times and finally electrodialyzed until free from chlorides. This material as obtained from peat or from soil corresponds to the corn cob lignin with respect to the method of preparation.

Bromination was carried out by treatment of the materials with a mixture of bromine and water at elevated temperatures in sealed glass tubes of the Carius type. The electric furnace used for heating was fitted with four iron

pipes in which the glass tubes were placed during treatment. An ordinary thermometer was used to indicate the temperature. The proportions of bromine and water, the temperature, and the time of treatment were varied during the course of the experiments.

The tubes containing the reaction mixtures were placed in the furnace at the desired temperature and at the completion of the treatment were left overnight to cool before being opened. Pressure was usually developed and was relieved by breaking off the capillary seal. The contents of each tube were transferred to a beaker with the aid of a stream of wash water. The diluted mixture in each case was filtered through an ordinary filter paper, thereby separating the soluble and insoluble fractions.

Bromine determinations were made by a modification of the Carius method. Digestion of the material with fuming nitric acid and conversion of the halogen to silver bromide were accomplished in small glass tubes heated to 300°C., in a special electric furnace recently described by Clark (1). The silver bromide was filtered by means of a Pregl tube and weighed in the usual manner.

Ether and alcohol extracts of the brominated materials were made by means of a Soxhlet extraction apparatus. Total nitrogen was determined by the Kjeldahl method, and ammoniacal nitrogen, by distillation with magnesium oxide.

The separation and the recrystallization of crystalline material resulting from bromination were carried out as described later in connection with the identification. Molecular weight determinations were made by the boiling method with acetone as the solvent. The apparatus used was that described by Lassar-Cohn (3, p. 322).

EFFECT OF VARIATION IN TEMPERATURE AND TIME OF TREATMENT OF PEAT WITH AQUEOUS BROMINE

The effect of a variation from 1 to 4 hours' heating with an excess of bromine at 100°, 125°, and 150°C. was investigated. One-gram samples of sawgrass peat were used with 10 cc. of water and 2 cc. of bromine. It was found necessary, however, to increase the quantity of bromine to 5 cc. for the treatment at 150°C., in order to maintain an excess.

The proportions of oven-dried soluble and insoluble matter were expressed as percentages calculated on the basis of the oven-dry weight of the original sample taken for treatment. The contents of bromine and nitrogen were expressed as percentages of the particular fractions analyzed.

As shown in table 1, an increased amount of material was found to have been rendered soluble by the bromination reaction with increased time of treatment at 100°C. At higher temperatures smaller differences were found in the percentages, with a slight tendency for the quantity of soluble matter to decrease with longer periods of heating. Continued heating at any one of the temperatures employed caused a marked reduction in the percentage of the insoluble fraction. This, however, did not cause a corresponding increase

in the soluble fraction, since a portion of the material was completely oxidized to carbon dioxide or other gaseous products. Complete oxidation of a portion of the organic matter was favored either by raising the temperature or by increasing the time of treatment as shown by the percentages of total yield of the brominated product. After 1 hour of treatment at 100°C., the total yield was 140.2 per cent, whereas after 4 hours' heating at 150°C., the corresponding value was only 94.9 per cent. In the latter case the loss of material by oxidation exceeded the weight of bromine absorbed by the reaction. Considerable pressure, also, was developed in the tubes during the treatment at 150°C. Nevertheless, the resistance of peat to the action of such a powerful oxidizing agent as bromine is worthy of note.

TABLE 1

Effect of variations in temperature and time of heating on a bromination reaction with sawgrass peat*

TEMPERATURE	TIME OF TREATMENT	MATERIAL DISSOLVED BY BROMINATION	MATERIAL REMAINING INSOLUBLE	TOTAL YIELD OF BROMINATED PRODUCT	BROMINE CONTENT		NITROGEN CONTENT		
					Of soluble matter	Of insoluble matter	Of soluble matter	Of insoluble matter	NH_3-N of soluble matter
°C.	*hours*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
100	1	53.1	87.1	140.2	36.9	39.5	4.00	1.30	
	2	54.8	84.8	139.6	37.1	39.9	4.05	1.25	
	3	57.3	75.2	132.5	38.9	44.5	4.54	0.89	
	4	59.5	70.9	130.4	40.7	47.7	4.56	0.75	
125	1	58.0	74.6	132.6	41.6	47.1	4.64	0.87	
	2	56.4	76.4	132.8	41.8	45.3	4.80	0.85	
	3	57.7	58.5	116.2	47.2	49.6	5.26	0.55	
	4	54.5	54.5	108.8	49.4	52.6	5.67	0.40	
150	1	54.7	58.3	113.0	50.4	62.8	5.81	0.49	3.50
	2	60.6	41.2	101.8	58.2	63.1	5.33	0.40	3.63
	3	60.0	38.5	98.5	56.8	65.8	5.79	0.22	3.85
	4	59.5	35.4	94.9	56.1	62.1	5.52	0.23	3.91

* A portion of the data in this table has previously been reported in a doctor's thesis (4).

The total content of bromine of both the soluble and insoluble fractions increased with increasing temperature and time of treatment except after continued heating at 150°C. The percentage of nitrogen, on the other hand, steadily diminished under the same conditions until only 0.23 per cent nitrogen remained in the insoluble fraction after 4 hours' treatment at 150°C. The untreated peat contained 3.37 per cent nitrogen, and on the basis of the original content approximately 97 per cent of the total was finally rendered soluble. Ammoniacal nitrogen, determined in the soluble fraction from treatment at 150°C., was found to comprise the major portion of the total nitrogen in this fraction. Continued treatment at 150°C., however, brought about only a relatively small increase in the percentage of ammoniacal nitrogen.

BROMINATION AND FRACTIONATION OF LIGNIN AND HUMIC ACIDS

In the extraction of the alkali-soluble material from the various samples for the preparation of the humic acid fraction it was found that 71.0 per cent of sawgrass peat was dissolved by 2 per cent sodium hydroxide but that only 55.5 per cent of sphagnum peat was rendered soluble (table 2). Addition of hydrochloric acid caused a portion of the alkali extract to precipitate but in varying proportions depending upon the source of the material. Lignin, of course, was entirely alkali-soluble and acid-insoluble because of the particular method of preparation. Only 24.7 per cent of the alkali extract from sphagnum peat was recovered as acid-insoluble (humic acids), whereas 54.2 per cent was obtained in the case of sawgrass peat.

TABLE 2

Solubility of various organic materials in 2 per cent NaOH and in dilute HCl

SAMPLE	SOLUBLE IN 2 PER CENT NaOH	PROPORTION OF NaOH-SOLUBLE MATERIAL INSOLUBLE IN HCl	YIELD OF HUMIC ACID BASED ON ENTIRE SAMPLE
	per cent	*per cent*	*per cent*
Sawgrass peat	71.0	54.2	38.5
Sphagnum peat	55.5	24.7	13.7
Soil organic matter	.....	28.8	.....
Corn cob lignin	100.0	100.0	100.0

TABLE 3

Fractionation of lignin and humic acids treated with bromine and water 3 hours at 110°C.

SOURCE	MATERIAL* DISSOLVED BY BROMINATION TREATMENT	MATERIAL* REMAINING INSOLUBLE	TOTAL YIELD† OF BROMINATED PRODUCT	INSOLUBLE FRACTION FROM BROMINATION			YIELD OF TETRABROMQUINONE
				Ether-soluble	Alcohol-soluble	Residue	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Corn cob lignin	25.5	74.5	146	90.3	9.6	0.0	10.4
Sphagnum peat	27.0	73.0	152	48.9	44.8	1.0	5.9
Sawgrass peat	30.6	69.4	154	39.8	32.7	23.8	4.2
Soil organic matter	53.3	46.7	121	28.9	39.8	31.3	1.0

* Expressed as percentage of the entire brominated product.

† Expressed as percentage of original sample taken for treatment.

The proportion of soil organic matter soluble in sodium hydroxide was not obtained, since the total content of organic matter in the soil sample was not determined. The yield of humic acids relative to the total alkali-soluble matter, however, was calculated after correction for inorganic salts in the acid filtrate from the humic acid precipitation. The latter was accomplished by treating the evaporated acid-soluble material with 30 per cent hydrogen peroxide and weighing the dried inorganic residue.

Treatment of the hydrochloric-acid-insoluble material or humic acid frac-

tion with bromine and water for 3 hours at 110°C., resulted in a product of which a large proportion remained insoluble in the reaction mixture. Considerable variation, however, was found in the relative quantities of insoluble matter after bromination of the different materials. The values ranged from 46.7 per cent in the case of humic acids from soil organic matter to 74.5 per cent in the case of corn cob lignin (table 3). The quantities of the soluble fraction varied similarly but in the reverse order.

The materials were arranged in table 3, as well as in the other tables, in the order of their degree of decomposition as found in their original or natural condition. This arrangement permits relationships to be seen more readily. Soil organic matter was considered as having been decomposed to the greatest extent, whereas corn cobs from which the lignin was isolated had undergone no decomposition. The peats were in an intermediate stage of decomposition.

The quantities of ether-soluble matter produced by bromination exhibited a regular decrease with increasing degree of decomposition with an accompanying but less regular increase in the alcohol-soluble material. There was also an increase in the material not soluble either in ether or in alcohol, amounting to as much as 31.3 per cent of the insoluble fraction from brominated soil humic acids.

SEPARATION AND IDENTIFICATION OF TETRABROMQUINONE

It was noted that the mixtures resulting from the bromination of lignin and humic acids contained a quantity of brilliant yellow platy crystals. The optimum conditions of formation appeared to be at temperatures of 105° to 110°C., with 25 cc. of water and 3 cc. of bromine per gram of sample. The time of treatment necessary was approximately 3 hours.

The crystalline material was not soluble in water and hence remained in the insoluble fraction. It was dissolved by ether, and when the evaporated ether extract was taken up with a small volume of 95 per cent alcohol the crystalline compound remained largely insoluble whereas the remaining material was dissolved. The crystals, however, were appreciably soluble in hot alcohol, which served as the solvent for their recrystallization. Those from lignin were obtained in the largest quantities and were capable of being purified the most readily.

The molecular weight, melting point, and elementary composition were determined. These values corresponded, within limits of experimental error, with values calculated (table 4) or obtained experimentally from pure tetrabromquinone. The latter was prepared for comparison from quinone by the method of Sarauw (15). Briefly, this consisted of the addition of an excess of bromine to a solution of quinone in glacial acetic acid. A good yield of tetrabromquinone separated out after the mixture had been kept hot for an hour or more. Recrystallization was accomplished in the same manner as with the compound obtained from lignin.

The melting point of both compounds, after several recrystallizations, was

found to be 295°C. (uncorrected). The total bromine content was 75.5 and 75.4 per cent, respectively, for the lignin compound and for the prepared tetrabromquinone. Crystals obtained from the peat humic acids, although possessing similar bromine contents, could not readily be purified to the extent that agreement in melting point was obtained. Optical examination by Mr. G. L. Keenan of the Food and Drug Administration, however, showed that the crystals from peat had indexes of refraction corresponding with those of the known compound.

Another property which served in confirming the identification of the crystals from lignin and humic acids as tetrabromquinone included the formation of a purple color when the compounds were dissolved in a warm solution of potassium hydroxide.

The yield of crystals from soil humic acids was very small, and no attempt was made to purify them sufficiently for analysis. They had the same characteristic appearance and gave the color test with potassium hydroxide, which left no doubt as to their identity.

TABLE 4

Analysis of tetrabromquinone as isolated from brominated lignin

CHARACTERISTIC	LIGNIN COMPOUND	CALCULATED FOR TETRABROMQUINONE
Carbon content.....*per cent*	17.63	16.98
Hydrogen content.....*per cent*	0.18	0.00
Bromine content.....*per cent*	75.50	75.45
Molecular weight.....	415*	423.7
Melting point.....*°C.*	295	298†

* Average of four determinations.

† As reported by Datta and Chaterjee (3).

The crystalline material as obtained from each sample was weighed in the impure condition before the first recrystallization, and the percentage yield was calculated on the basis of the weight of the entire brominated sample. These results, although not highly accurate, are comparative and serve to distinguish the different types of organic matter. The percentages (included in table 3) show the greatest yield for lignin and the least for soil humic acids.

Oxalic acid was found in the soluble fractions from bromination, but since this compound is commonly produced in oxidation reactions no conclusions could be drawn from the formation of this substance relative to differences in character of the humic acids.

BROMINE CONTENT OF VARIOUS FRACTIONS OF BROMINATED LIGNIN AND HUMIC MATERIAL

The bromine content of the insoluble fraction from the bromination treatment was greatest in the case of lignin and least in the humic acids from soil organic matter as shown in table 5. The reverse was true of the soluble

fraction, although smaller differences were noted in the analyses of the latter fractions.

In order to obtain some information relative to the nature of the combined bromine, small samples of the insoluble fraction (0.2 gm.) were boiled 5 minutes with 15–20 cc. of a 5 per cent solution of sodium hydroxide in absolute alcohol. The mixtures were then diluted to approximately 75 cc. with water, acidified with nitric acid, and filtered to remove all precipitated organic matter. Silver nitrate was then added, and the silver bromide was determined in

TABLE 5

Bromine content of various fractions of brominated lignin and humic acids

SOURCE	INSOLUBLE FRACTION FROM BROMINATION			TOTAL Br OF SOLUBLE FRACTION FROM BROMINATION
	Total Br	Br split off by alcoholic NaOH	Proportion of total Br split off by alcoholic NaOH	
	per cent	*per cent*	*per cent*	*per cent*
Corn cob lignin	64.0	38.2	59.7	43.5
Sphagnum peat	54.8	41.7	76.0	37.5
Sawgrass peat	53.4	36.2	67.8	44.7
Soil organic matter	40.0	34.4	86.0	47.7

TABLE 6

Nitrogen content of various fractions of brominated lignin and humic acids

SOURCE	N CONTENT OF UNTREATED HUMIC ACID	INSOLUBLE FRACTION FROM BROMINATION			SOLUBLE FRACTION FROM BROMINATION			
		Total N	Ether-soluble N	Alcohol-soluble N	Total N	Total N calculated as proportion of original N	NH_3-N	NH_3-N calculated as proportion of soluble N
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Corn cob lignin	1.80	0.38	0.50	2.63	4.15	86.1	1.65	39.8
Sphagnum peat	2.39	0.98	0.54	1.62	3.80	65.2	1.51	39.7
Sawgrass peat	3.92	1.62	0.95	2.70	4.86	58.4	1.95	40.1
Soil organic matter	4.90	2.12	1.06	2.45	5.76	75.8	2.68	46.5

the usual manner. Only the readily ionizable bromine is obtained by this method, since that linked to an aromatic nucleus is not split off by the alcoholic sodium hydroxide. The results as recorded in table 5 indicate that nearly 60 per cent of the bromine in the brominated lignin is split off as compared with 86 per cent of the brominated soil humic acids.

NITROGEN DISTRIBUTION

The nitrogen contents of the untreated lignin and humic acid fractions were quite different, as shown in table 6. The humic acids from soil organic mat-

ter contained nearly 5 per cent nitrogen, whereas humic acids from the less decomposed materials contained less nitrogen. Lignin contained 1.80 per cent nitrogen, the least of any of the materials.

Most of the nitrogen of the different materials was rendered soluble by the bromination treatment; to this extent there was some degree of similarity in the behavior of the nitrogenous constituents. The proportional amounts of the total nitrogen which were dissolved varied from 58.4 per cent for the humic acid fraction from sawgrass peat to 86.1 per cent for lignin. The corresponding percentage for soil humic acids was 75.8, which was not greatly different from the value for lignin.

The quantities of nitrogen remaining insoluble possessed the same relative order of magnitude as the nitrogen contents of the untreated materials (table 6). This relationship held also for the nitrogen of the ether extracts. The alcohol-soluble materials contained irregular percentages of nitrogen.

From 39.7 to 46.5 per cent of the nitrogen found in the soluble fraction was ammoniacal in form. This variation, however, was not sufficiently wide to permit definite distinctions to be made between the different materials relative to the character of the soluble nitrogen.

SUMMARY AND CONCLUSIONS

The lignin and humic acid preparations studied in this investigation had similar properties to the extent that they were each soluble in sodium hydroxide solution and were precipitated by dilute hydrochloric acid. The bromination treatment, however, has served to distinguish sharply not only between the lignin as compared with the humic acids but also among the individual humic acid fractions.

The conditions of bromination were arbitrarily fixed as to quantity of bromine and water, temperature, and time of treatment. A variable product results from the treatment under varying conditions as shown in the case of a sample of peat.

Differences in character of the different preparations were most clearly and consistently illustrated by the proportions of organic matter dissolved during the bromination treatment, by the relative quantities of ether-soluble matter produced, and by the relative yields of crystalline tetrabromquinone. The comparative ease with which combined bromine is removed by treatment with alcoholic sodium hydroxide was also shown to be a distinguishing characteristic.

The untreated lignin and humic acids contained widely different percentages of nitrogen, but some degree of similarity was shown in the proportions of the total nitrogen dissolved during the bromination treatment and in the character of the soluble nitrogen with respect to the ammoniacal form. The percentages of insoluble nitrogen, however, exhibited variations in the same relative order as did the nitrogen contents of the untreated samples.

Evidence of the presence of aromatic constituents in the composition of

lignin, and to a lesser degree in the humic acid preparations, was found in the production of tetrabromquinone. The presence of aromatic groups is in accord with the majority of formulas proposed for lignin (14).

The chemical behavior of the humic acid preparations toward bromination appeared to vary according to the degree of decomposition of the material from which these fractions were separated. Comparisons with the behavior of lignin indicated, in general, that the humic acid fraction becomes less similar to lignin as decomposition advances. It is of course recognized that lignin from different sources varies in composition and that other comparisons might have shown somewhat different relationships.

If the formation of tetrabromquinone is at all characteristic of lignin under the conditions of the bromination treatment it may be concluded from the relative yields as well as from the other relationships already discussed that lignin comprises only a small part of the humic acid fraction derived from partly decomposed peat or soil organic matter. Lignin, if the type found in corn cobs was originally present in the plant material, must, therefore, either have undergone extensive decomposition or have lost its identity in the formation of new complexes (19) which comprise the humic acid fraction.

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RECLAMATION OF ALKALI SOILS BY ELECTRODIALYSIS

AMAR NATH PURI AND BALMOKAND ANAND

Irrigation Research Institute, Lahore, India

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The application of the principle of electrodialysis to the removal of exchangeable bases from soils is well known. It is based on the fact that soil particles, being negatively charged, move toward the anode, and exchangeable bases, toward the cathode. If the particles in a wet soil block are not free to move, then it is the water that moves, and we have the phenomenon of electrosmosis. As a matter of fact in soils we are dealing both with electrodialysis and electrosmosis.

It is well known that alkali soils owe their characteristic features to the presence of exchangeable sodium, and improvement in such soils is accompanied by a marked reduction in the latter. The underlying principle of all the methods of reclaiming alkali soils is the removal of exchangeable sodium, which can be brought about by treatment with a Ca salt or an acid.

The application of electrodialysis on a field scale is a novel method which holds vast possibilities. Not only are no extraneous chemicals required, but the by product is sodium hydroxide, which is of economic value. A very important feature of the process is the increased rate of percolation and opening up of the soil. The rate of percolation increases as soon as the electric current is switched on and continues long after it is switched off. This residual effect is of great importance and the one likely to prove most useful in reducing the cost of reclamation.

The present investigation was divided into two parts, one dealing with laboratory experiments and the other with field trials.

LABORATORY EXPERIMENTS

A large Buchner funnel 18 inches in diameter was filled with a highly alkaline soil of pH value 11. The usual porcelain sieve of the funnel was replaced by a perforated brass plate and formed the cathode. Five cylindrical anodes were arranged symmetrically at the top along the circumference. A layer of water 1.5 inches deep was maintained on top of the soil. The wet soil was absolutely impervious to water: not a drop came out in 24 hours.

A current of 1.5 amperes with a terminal potential of 20 volts was allowed to pass through the soil. After a few minutes the water began to trickle in drops and soon formed a thin stream. After 4 hours, the pH value of the water coming out was 12.62. The current was stopped; the rate of percolation decreased

but still remained appreciable. After 36 hours, the pH value of the soil in the vicinity of the anodes was 8.66, while the soil in the top center nearer the cathode was 10.70. Once again in the same soil a current of 1.5 amperes was passed for 2 hours, and the cathode liquid was collected and titrated for alkalinity. It was found to be 0.33 *N*, which became 0.4 *N* after another hour. The current was then increased to 2.4 amperes, and within an hour the normality of the percolate went up to 0.6 *N*. The current was then stopped, and the percolate was collected overnight. The alkalinity of the liquid collected next morning was 0.5 *N*. This alkali was 90 per cent NaOH and the rest Na_2CO_3. The head of water at the top of soil was maintained at about 1.5 inches by adding fresh water, and leaching went on for several days without any current. The pH values of the different portions of the soil on the second day were as follows: anodic space, 5.53; interspaces between anodes, 10.11; cathode space, 13.28.

Another experiment in which a soil of pH 10.5 was used gave an equivalent of 13 gm. of NaOH in 5 hours with a current of 2.5 amperes and a terminal potential of 10 volts. After the current was shut off, another 4 gm. of NaOH was collected in the percolate during 14 hours. The anodes in this case were plates of ordinary iron and rustless steel. They were eaten away to the extent of 50 per cent and 25 per cent respectively. Ordinarily the cost of replacing electrodes would not be a matter of serious consideration, as any scrap iron could be used and an iron salt left in an alkali soil would be rather an advantage.

Another experiment was arranged in a wood box 2 by 2 by 3 feet. Five types of cathodes, as follows, were used:

A. Iron pipe 2 feet long perforated all around.
B. Iron pipe 2 feet long perforated on the upper surface only.
C. V-shaped wood pipe 2 feet long covered with thin perforated iron sheet.
D. V-shaped wood pipe covered with perforated brass sheet.
E. Porous tube containing tubular wire-gauze as electrode.

A current of 6 amperes was run for 3 hours with a terminal potential of 33 volts. The total current consumed was 0.6 unit. Discharge of alkali was as follows:

A = 250 cc. of 0.2 *N* solution
B = 200 cc. of 0.25 *N* solution
C = 115 cc. of 0.2 *N* solution
D = 200 cc. of 0.2 *N* solution
Total NaOH 6.6 gm.

Percolation in E was very small and consequently was not recorded. Unlike the others, the percolate, however, was absolutely clear.

The current was next increased to 10.5 amperes with a terminal potential of 50 volts. The energy consumed was 1 unit. The amount of alkali collected in 2 hours was as follows:

A = 250 cc. of 0.33 *N* solution
B = 250 cc. of 0.36 *N* solution
C = 225 cc. of 0.28 *N* solution
D = 250 cc. of 0.3 *N* solution
Total NaOH = 12.7 gm.

Percolation went on during the night without any current. Next morning 700 cc. of 0.25 *N* alkali was collected from all the four electrodes.

A current of 14 amperes was next passed for 4 hours with a terminal potential of 66 volts. The energy consumed was 3.7 units. The following quantities of alkali solution were obtained:

A = 950 cc. of 0.33 *N* solution
B = 600 cc. of 0.36 *N* solution
C = 500 cc. of 0.33 *N* solution
D = 400 cc. of 0.36 *N* solution
Total NaOH = 34 gm.

The most important finding from the laboratory studies was the increased rate of percolation which continued even after the current was switched off.

FIELD EXPERIMENTS

A plot of land 15 by 15 feet was trenched all around. The trench was 3.5 feet deep and 1 foot wide. The cathode was an iron tube 6 feet long and 4 inches in diameter with perforations at the top. This was buried in the soil to a depth of 3 feet by digging a furrow from the center of the plot toward the middle of the trench. It had a gentle slope toward the trench to ensure easy flow of water. A ridge about 6 inches high was made all around the plot to ensure a certain head of water. The trench was filled with water, and a liberal watering was given at the top to bring about the initial saturation of the soil, after which the water from the trench was pumped out. The anode was a big sheet of iron 3 by 6 feet long laid at the top of the moist soil block just above the cathode. After the initial saturation the plot was watered to a depth of 4 inches. No attempt was made to keep the head of water constant, as the experiment did not last long enough to necessitate that precaution. A vessel was put beneath the protruding end of the cathode in the trench to find the initial rate of percolation. It was practically nil. A current of 22 amperes from a 220-volt main was passed through a suitable resistance. Alkali began to trickle almost immediately and soon formed a thin stream; 2.5 liters were collected in 45 minutes. The strength of the solution was 0.1 *N*. Rains intervened, and the experiment had to be discontinued. One year after, the same electrodes were connected again. During the intervening period the cathode had become choked, and, therefore, when the current was switched on the alkali did not come out until after an hour and even then the rate of percolation was only 300 cc. an hour, which fell to 50 cc. an hour when the current was switched off. The increase in the rate of percolation was very marked and was tested several times by switching the current on and off. The strength of

the alkali coming out was 0.05 *N* against 0.1 *N* obtained the previous year. The soil, however, had been considerably improved by the first treatment and the rains that followed. There was a remarkable decrease in the exchangeable sodium in the surface 3 inches. To start with it had 4.8 m.e. per 100 gm., which fell to 3.5 after 2 hours' treatment and to 2.8 on further treatment for 4 hours. The total current consumed was 5 units.

Next, a field 17 by 50 feet was selected and trenched all around. The anode was a sheet of iron; and the cathode, a twisted wire rope about 1 inch in diameter (a rejected rope of a steam tackle), was laid in the drain on three sides. The plan of the experiment is shown in figure 1.

A current of 22 amperes with a terminal potential of 153 volts was passed through. When the anode was in position *a*, the total resistance was 7 ohms; at positions *b* or *c*, it was 9 ohms. When the area of the anode was increased

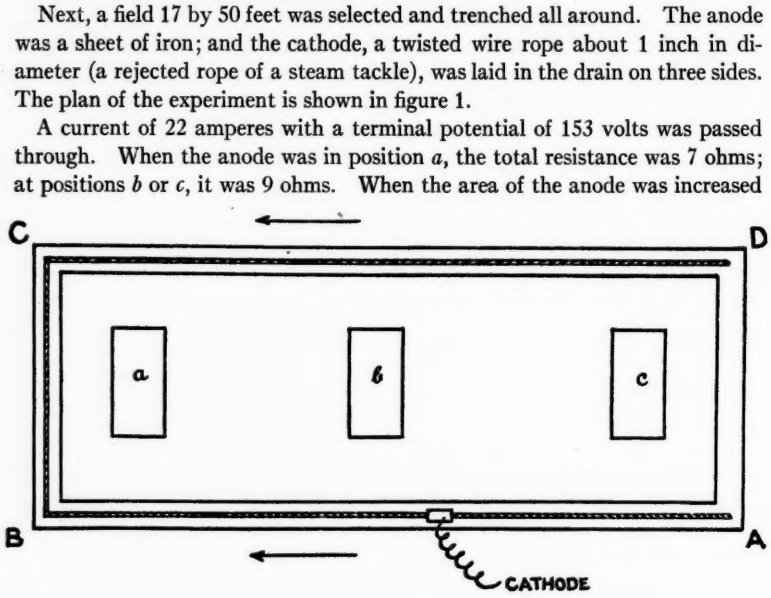

FIG. 1. ELECTRO-RECLAMATION OF SOIL—PLAN OF FIELD EXPERIMENT II

about five times, the resistance fell to 2 ohms. Increasing the area of the cathode did not have very appreciable effect on resistance.

A good deal of water was standing in the drain B-C, and in 2 hours the alkalinity of the water rose by 1 m.e. per liter. Because of a slope, there was not much water in the drains A-B and C-D. The alkalinity of a small amount of water collected in a puddle had increased by 6 m.e. per liter as a result of the passage of the electric current. The alkalinity of the water around the anode decreased by 1.3 m.e. per liter. During 5 hours' electrodialysis the soil had lost per 100 gm. 0.6 m.e. of exchangeable sodium in the top 3 inches.

DISCUSSION OF RESULTS

It is not possible at this stage to devote much space to the discussion of results. Facts are presented merely to illustrate the possibility of applying electrodialysis to the reclamation of alkali soils. Before starting these experiments, the authors had grave misgivings as to the success of the method. It

was argued that the soil would act as an infinite conductor, and the whole of the electrical energy would be diffused. Practical trials, however, proved this supposition to be incorrect. The method, it seems, holds out promise of practical importance in other directions as well. During the process of electro-reclamation, phosphates and nitrates are likely to move to the upper surface where they will prove useful to plants. This point and the effect on the micro-biological population of the soil are being studied. The application of gypsum together with electric treatment is likely to prove more beneficial than without such treatment. A preliminary experiment in this direction showed a marked decrease in electrical resistance when gypsum was applied.

The economics of electro-reclamation have not been studied thoroughly. They must depend largely on local conditions, such as the cost of electricity and the value of the land, and must be worked out by individual workers themselves. In the light of our experience, however, we can offer the following suggestions, which might prove helpful to others who may like to give a trial to electro-reclamation:

The resistance of the soil block should not exceed 1 ohm. This was accomplished in our soil with a clay content of about 15 per cent, in a plot of land 50 by 50 feet. The most important factor in this connection is the size of the anode, which can be made out of large sheets of scrap iron. Soils with a heavier texture will require a larger anode than lighter ones.

The terminal potential should be low and the amperage high. We suggest a generator of 25 volts and 250 amperes.

Electric current should not be passed continuously for a long time. It is more advantageous to pass the current for 5 to 6 hours and then allow an interval of about the same time or longer.

SUMMARY

The well-known principle of electrodialysis for the removal of exchangeable bases has been applied to the reclamation of alkali soils.

Preliminary experiments have been described which show that exchangeable sodium as NaOH can be removed from soil on a field scale by the application of electric current, resulting in a marked reduction in the exchangeable sodium of the soil thus treated.

THE EFFECT OF CALCIUM IONS AND REACTION UPON THE SOLUBILITY OF PHOSPHORUS[1]

E. J. BENNE, A. T. PERKINS AND H. H. KING

Kansas Agricultural Experiment Station

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The literature on the subject of the fixation and availability of phosphorus is voluminous though frequently contradictory. The synonymous use of "availability" and "solubility" has led to much confusion. Statements made concerning the tendency of a cation to precipitate phosphorus from solution without specifying the existing pH or the other cations or anions present have conflicted with statements on the same subject by other investigators working under slightly different conditions.

No attempt will be made in this paper to give a complete review of the literature pertaining to the subject. To do so adequately would require the compilation of an extensive bibliography.

Recent investigations of a closely related nature have been conducted by: Gaarder (4), Austin (2), Teakle (14), Patten (11), Osugi, Hoshie, and Nishigaki (9), McGeorge and Breazeale (8), Heck (6), Sanfourche and Henry (12), Ford (3), Antonov (1), and Spurway (13).

The investigation described in this paper is based largely upon the work of Gaarder (4), with some variations, however, in the nature and treatment of the systems used. The authors, as all workers in this field should be, are appreciative of Gaarder's excellent work, and in so far as the procedures used by them correspond to those of Gaarder, the results obtained corroborate his. Unquestionably the work of many others is fully as valuable as that of those listed but is perhaps not so specifically related to this investigation.

EXPERIMENTAL PROCEDURE AND RESULTS

The results reported in this paper are from a study of the solubility of phosphorus in dilute solutions of H_3PO_4 when treated with varying amounts of different calcium compounds or of a soil saturated with calcium, as the pH of the systems was varied by different means. The mixtures, contained in stoppered bottles of suitable size, were agitated continuously for 24 hours in a water bath at 25°C. At the end of the agitation period the contents of the bottles were filtered as rapidly as possible, and the amounts of calcium and phosphorus in the filtrates and their pH values were determined by standard methods. Water free from CO_2 was used throughout the work.

[1] Contribution No. 203 from the department of chemistry. Data from a thesis to be presented as partial fulfilment of the requirements for the degree of doctor of philosophy. Published with the consent of the Graduate Council.

TABLE 1

Effect of increasing amounts of $CaCO_3$ upon phosphorus solubility in a dilute solution of H_3PO_4

BOTTLE NUMBER	Ca ADDED*	pH OF FILTRATE	Ca IN SOLUTION	P IN SOLUTION
	gm.		*gm.*	*gm.*
1	0.0511	4.88	0.0511	0.0791
2	0.1021	5.39	0.0536	0.0791
3	0.1532	5.52	0.0538	0.0785
8†	0.7660	5.89	0.0590	0.0782
9	1.1490	6.59	0.0345	0.0184
10	1.5320	6.62	0.0361	0.0092
14	4.5960	7.01	0.0401	0.0064

* Added to 250 cc. of solution containing 0.0791 gm. of P as H_3PO_4.

† Several points were established between bottles 8 and 9. The data were destroyed by fire and were not repeated since they showed nothing unusual.

TABLE 2

Effect of a constant amount of calcium ion, added in varying proportions of $CaCO_3$ and CaO, upon phosphorus solubility in dilute solutions of H_3PO_4

BOTTLE NUMBER	Ca AS CaO*	Ca AS $CaCO_3$	pH OF FILTRATE	Ca IN FILTRATE	P IN FILTRATE
	gm.	*gm.*		*gm.*	*gm.*
1	0.0000	0.3064	5.67	0.0548	0.0786
2	0.0511	0.2554	6.59	0.0264	0.0185
3	0.1021	0.2043	7.08	0.0227	0.0013
4	0.1532	0.1532	7.36	0.0011	0.0000
5	0.2043	0.1021	11.05	0.0259	0.0000
6	0.2554	0.0511	11.78	0.0529	0.0000
7	0.3064	0.0000	12.14	0.0781	0.0000

* Added to 250 cc. of solution containing 0.0791 gm. of P as H_3PO_4.

TABLE 3

Effect of a constant amount of $CaCO_3$ (containing 0.3064 gm. of Ca) upon phosphorus solubility in dilute solutions of H_3PO_4 when the reaction of the system was varied with NaOH or HCl*

BOTTLE NUMBER	0.15 *N* HCl ADDED	0.3 *N* NaOH ADDED	pH OF FILTRATE	Ca IN FILTRATE	P IN FILTRATE
	cc.	*cc.*		*gm.*	*gm.*
2	100	0	2.28	0.3064	0.0791
3	75	0	5.09	0.2814	0.0778
4	50	0	5.36	0.2087	0.0779
5	25	0	5.51	0.1324	0.0781
8	0	0	5.79	0.0563	0.0779
9	0	1.25	6.06	0.0517	0.0704
10	0	2.50	7.14	0.0185	0.0108
13	0	10.00	7.84	0.0018	0.0117
14	0	20.00	10.04	0.0000	0.0359
15	0	30.00	11.21	0.0000	0.0188

* Added to 250 cc. of solution containing 0.0791 gm. of P as H_3PO_4.

The pH values below neutrality were determined by the quinhydrone electrode, and those above, by the hydrogen electrode. Calcium and phosphorus were determined by methods approved by the Association of Official Agricultural Chemists. Phosphorus was determined colorimetrically in some of the filtrates which yielded no precipitate of ammonium phosphomolybdate. Less than 0.5 p.p.m. was found in such cases. In most instances the amount in

TABLE 4

Effect of increasing amounts of CaO upon phosphorus solubility in dilute solutions of H_3PO_4

BOTTLE NUMBER	Ca ADDED*	pH OF FILTRATE	Ca IN FILTRATE	P IN FILTRATE
	gm.		*gm.*	*gm.*
2	0.0511	4.36	0.0511	0.0791
3	0.0574	5.50	0.0552	0.0784
4	0.0638	5.35	0.0462	0.0709
6	0.1021	5.47	0.0259	0.0409
7	0.1276	5.50	0.0137	0.0235
8	0.1404	5.92	0.0085	0.0135
9	0.1532	5.94	0.0039	0.0043
18	0.3064	12.21	0.0781	0.0000

* Added to 250 cc. of solution containing 0.0791 gm. of P as H_3PO_4.

TABLE 5

Effect of a constant amount of $CaCl_2$ (containing 0.2043 gm. of Ca) upon phosphorus solubility in dilute solutions of H_3PO_4, when the reaction of the system was varied with NaOH*

BOTTLE NUMBER	0.15 *N* NaOH ADDED	pH OF FILTRATE	Ca IN FILTRATE	P IN FILTRATE
	cc.		*gm.*	*gm.*
6	9.0	5.09	0.2043	0.0791
7	9.5	5.05	0.1978	0.0749
8	10.0	4.82	0.1889	0.0706
9	12.5	5.07	0.1568	0.0408
10	15.0	5.38	0.1431	0.0403
11	20.0	5.44	0.0955	0.0188
12	25.0	5.48	0.0528	0.0024
13	26.0	5.73	0.0415	0.0005
14	28.0	5.91	0.0320	0.0001
15	29.0	7.36	0.0263	0.0000
20	75.0	12.43	0.0018	0.0000

* Added to 250 cc. of solution containing 0.0791 gm. of P as H_3PO_4.

250 cc. of filtrate was less than 0.1 mgm.; hence, solubility in all such cases has been listed merely as 0.0000 gm.

Throughout the work, unless an exception is cited, each addition of calcium was made to 0.25 gm. of H_3PO_4 (0.0791 gm. of P) diluted to 250 cc. Likewise the amounts listed in the tables are based upon 250 cc. of filtrate. Certain insignificant data were omitted for the sake of brevity, as indicated by bottle numbers missing from the tables.

In the tables are summarized the nature and amounts of substances added and the results obtained. Graphical representations of the results appear in figures 1, 2, and 3.

The failure of $CaCO_3$ to precipitate the phosphorus completely, as indicated in table 1 and in figures 1 and 2, appeared to be due either to its slight solubility or to its moderate effect upon the reaction, or both. This suggested its use with CaO (table 2, fig. 2) and with NaOH (table 3, fig. 2), in which

TABLE 6

Effect of increasing amounts of a soil saturated with calcium upon phosphorus solubility in dilute solution of H_3PO_4

BOTTLE NUMBER	Ca ADDED*	pH OF FILTRATE	Ca IN FILTRATE	P IN FILTRATE
	gm.		*gm.*	*gm.*
1	0.0511	3.21	0.0301	0.0632
2	0.1021	3.99	0.0336	0.0586
3	0.1532	4.53	0.0329	0.0548
4	0.2043	4.84	0.0305	0.0495
5	0.2553	4.99	0.0281	0.0453
6	0.3064	5.27	0.0253	0.0406
7	0.3575	5.37	0.0246	0.0363
8	0.4085	5.50	0.0224	0.0324
9	0.4596	5.60	0.0213	0.0292
10	0.6128	6.32	0.0177	0.0185
11	0.7660	6.81	0.0175	0.0133
12	0.9192	7.06	0.0169	0.0098
13†	0.6128	6.48	0.0213	0.0138
14	0.7660	6.72	0.0318	0.0138
15	1.0724	6.85	0.0388	0.0075
16	1.2256	6.98	0.0375	0.0063
17	1.3788	7.02	0.0375	0.0038
18	1.5320	7.12	0.0350	0.0025

* Calculated as replaceable Ca^{++} and added to 250 cc. of solution containing 0.0791 gm. of P as H_3PO_4.

† Beginning of greater dilution. Added to 2500 cc. of solution containing 0.0791 gm. of P as H_3PO_4.

case the amount of calcium in solution could be varied or it could be kept constant and the reaction varied. CO_2 released from the $CaCO_3$ by the action of H_3PO_4 appeared to exert a marked effect upon the results in the foregoing experiments; hence CaO was also used alone (table 4, figs. 1 and 2). Since CaO is only moderately soluble and affects the reaction strongly, it was considered of interest to try the effect of $CaCl_2$, a freely-soluble though neutral salt. The results of this experiment are not tabulated here; however, it is significant to note that calcium added in this form in amounts ranging from

$\frac{1}{3}$ to 30 times the amount necessary to form $Ca_3(PO_4)_2$ failed to produce a precipitate, and the pH remained practically constant at slightly above 2. When the reaction of such systems was varied with NaOH, however, very

TABLE 7

Effect of a constant amount of soil saturated with calcium (containing 0.2553 gm. of replaceable calcium) upon phosphorus solubility in dilute solutions of H_3PO_4, when the pH of the system is varied with HCl, NaOH, CaO, or $CaCO_3$*

BOTTLE NUMBER	HCl ADDED	0.3 *N* NaOH ADDED	CaO ADDED	$CaCO_3$ ADDED	pH OF FILTRATE	Ca IN FILTRATE	P IN FILTRATE
	cc.	*cc.*	*gm.*	*gm.*		*gm.*	*gm.*
1	20†	0	0	0	0.02	0.2519	0.0786
2	15	0	0	0	0.29	0.2511	0.0755
3	10	0	0	0	0.39	0.2457	0.0690
4	125	0	0	0	1.96	0.2236	0.0342
5	75	0	0	0	2.56	0.1885	0.0220
6	25	0	0	0	3.74	0.0892	0.0303
7	0	0	0	0	5.02	0.0275	0.0428
8	0	10	0	0	6.31	0.0073	0.0424
9	0	25	0	0	7.57	0.0051	0.0366
10	0	40	0	0	7.73	0.0031	0.0229
11	0	55	0	0	8.00	0.0031	0.0124
12	0	0	0.0357	0	5.83	0.0255	0.0349
13	0	0	0.0714	0	6.27	0.0243	0.0295
14	0	0	0.1428	0	6.68	0.0188	0.0103
15	0	0	0.2142	0	7.22	0.0133	0.0022
16	0	0	0.2856	0	7.29	0.0120	0.0020
17	0	0	0.4284	0	7.46	0.0167	0.0000
18	0	0	0.6426	0	8.91	0.0187	0.0000
19	0	0	0.8568	0	9.21	0.0379	0.0000
20	0	0	0	0.3827	6.39	0.0326	0.0408
21	0	0	0	0.7654	6.59	0.0343	0.0392
22	0	0	0	1.9135	6.62	0.0356	0.0363
23	0	0	0	3.8270	6.72	0.0359	0.0347
24	0	0	0	7.6540	6.77	0.0356	0.0306
25	0	0	0	11.4810	6.89	0.0358	0.0277
26	0	0	0	15.3080	6.93	0.0332	0.0244
27	0	0	0	19.1350	6.99	0.0360	0.0240

* Added to 250 cc. of solution containing 0.0791 gm. of P as H_3PO_4.

† In bottles 1 to 3, concentrated HCl was used; in bottles 4 to 6, 0.15 *N* HCl was used.

pronounced effects upon phosphorus solubility occurred, as shown in table 5 and figure 2. In order to determine whether the principles indicated in the preceding work would hold with the more complicated soil systems, a soil

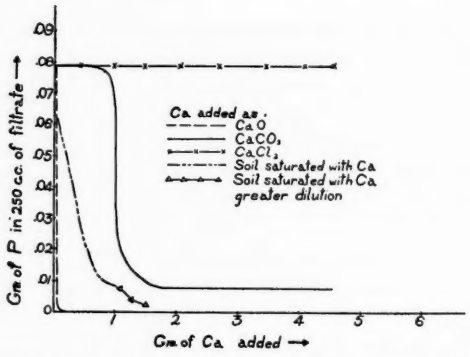

FIG. 1. EFFECT OF INCREASING AMOUNTS OF CALCIUM ION, ADDED IN DIFFERENT COMPOUNDS, UPON THE SOLUBILITY OF PHOSPHORUS IN A DILUTE SOLUTION OF H_3PO_4

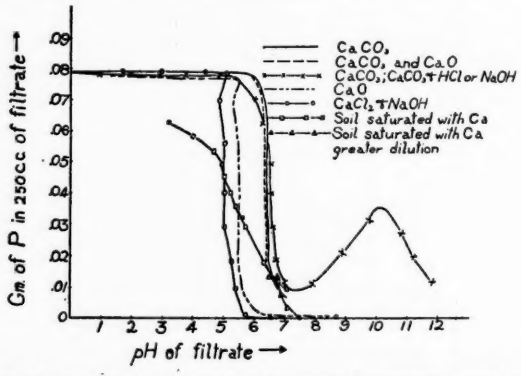

FIG. 2. EFFECT OF CALCIUM ION, ADDED IN DIFFERENT COMPOUNDS, UPON THE SOLUBILITY OF PHOSPHORUS IN A DILUTE SOLUTION OF H_3PO_4 AS THE pH IS VARIED BY THE CALCIUM COMPOUND ITSELF OR BY THE ADDITION OF ACIDS OR BASES

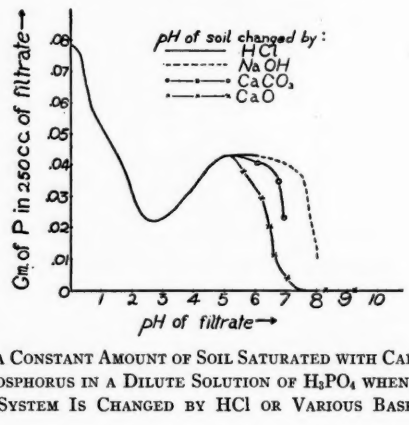

FIG. 3. EFFECT OF A CONSTANT AMOUNT OF SOIL SATURATED WITH CALCIUM UPON THE SOLUBILITY OF PHOSPHORUS IN A DILUTE SOLUTION OF H_3PO_4 WHEN THE pH OF THE SYSTEM IS CHANGED BY HCl OR VARIOUS BASES

saturated with calcium was subjected to similar treatment, both alone (table 6, fig. 2) and when the reaction was varied by HCl, NaOH, CaO, or $CaCO_3$ (table 7, fig. 3).

A quantity of Wabash loam soil was leached with normal calcium chloride solution until the leachate no longer yielded a positive test for the Mg ion. The excess $CaCl_2$ was removed by washing with distilled water. Replaceable calcium was determined by Gedroiz's method (5) and was found to comprise 20.38 m.e. per 100 gm. of soil. On the basis of replaceable calcium, sufficient soil was used to yield the different amounts of calcium listed in tables 6 and 7. In table 6, bottles 1–12 contained 0.25 gm. of H_3PO_4 in 250 cc. of solution; however, the large bulk of soil made it impossible to carry the work very far with this dilution. In order to extend the work and also to determine the effect of greater dilution upon the removal of phosphorus from solution, a dilution ten times as great was used in bottles 13–18.

DISCUSSION OF RESULTS

From the data, especially that with $CaCl_2$ alone, it is evident that Ca^{++} ions do not precipitate PO_4^{---} ions from solution, even in the presence of a high concentration of Ca^{++} ions, so long as the pH of the solution remains sufficiently low. This, of course, is a fact of importance in analytical work, as indicated by Patten's work (11). The results presented in tables 1, 2, 3, 4, and 5 and their graphical representations in figures 1 and 2 show that appreciable precipitation from these solutions did not begin until the pH approached 5.5, and maximum precipitation, or minimum solubility, did not occur until the pH approached 7.5. Although the minimum solubility of phosphorus varied considerably with the various combinations, its solubility remained almost constant at all higher pH values in the range covered, except in the work with $CaCO_3$ and NaOH, where the solubility rose somewhat, possibly because of an increase in the Na^+-ion concentration. The variations observed here are probably due to the presence of anions and cations which might be avoided in carefully controlled analytical conditions but which commonly occur in the soil.

The work with $CaCO_3$ shows that it did not reduce the phosphorus in solution to 0.0000 gm. during the 24-hour agitation period, although an enormous excess of $CaCO_3$ compared to phosphorus was present. This appears to be due to the slight solubility of the $CaCO_3$ and its moderate effect upon the pH of the solution. However, when the pH was raised with NaOH, $CaCO_3$ still did not cause complete precipitation of phosphorus, a fact which indicates that its slight solubility is a major factor in its failure to do so. This was perhaps partly due to the effect of the unlike Na^+ ions; however, there were apparently not enough Ca^{++} ions to overcome this effect and to precipitate completely the phosphorus, as occurred when soluble $CaCl_2$ was used.

It was of interest to notice that when the CO_3^{--} ion was present there was a tendency toward supersaturation of the solutions with respect to calcium phosphate. Often a short time after filtration the filtrates became cloudy

and calcium phosphate precipitated; hence, in order to obtain consistent results, it was imperative to withdraw the aliquots for analysis soon after filtering.

The work with mixtures of $CaCO_3$ and CaO and with CaO alone shows that the solubility of CaO and its effect on the pH were great enough to cause it to reduce the phosphorus content of the filtrate to 0.0000 gm. when only slightly more than enough to form $Ca_3(PO_4)_2$ with the H_3PO_4 present had been added. Figures 1 and 2 illustrate these differences. The results recorded in table 4 are of especial interest since through the use of CaO and H_3PO_4 no cations or anions other than Ca^{++} and PO_4^{---} were introduced to vary the H^+- and OH^--ion concentration.

Work with mixtures of CaO and $CaCl_2$ and with NaOH indicates that the pH of the filtrate became less after some precipitation had occurred than it was at the point where precipitation first began, in spite of the presence of additional increments of the base. Such behavior certainly appears anomalous; however, it has been observed before. Wendt and Clarke (15) ascribe it to the transitory nature of $CaHPO_4$ and to slowness in the establishment of a stable equilibrium. It could be due to differences in the degree of ionization of the acid phosphate.

It is of interest to notice in table 4 how the calcium in solution increased until the phosphorus began to precipitate, then decreased to a minimum with the phosphorus but rose again after all of the phosphorus had been withdrawn from solution.

Figures 1 and 2 show exceptionally well how valueless is a statement concerning the precipitation of phosphorus from solution by Ca^{++} ions or any other ions without consideration of the effect of anions present or of the pH of the system concerned.

The values recorded in table 6 show that the soil saturated with calcium acted somewhat as a sparingly soluble calcium salt from a weak acid in its precipitating effect upon phosphorus. Increasing amounts raised the pH of the filtrate and decreased the phosphorus in solution much as did increasing amounts of $CaCO_3$. It will be noted, however, that the curve for the soil is below that for $CaCO_3$, very probably because of adsorption by the large bulk of soil. Greater dilution appeared to have no pronounced additional effect upon phosphorus in solution or upon pH value but, as might be expected, it materially increased the amount of calcium in solution.

The data in table 7 and their graphical representation in figure 3 show very significant variations in the amounts of phosphorus in extracts from a soil saturated with calcium when the reaction was changed with HCl or different bases. When the pH of the system was sufficiently low, essentially all of the added phosphorus remained in solution; apparently, even that which would normally be adsorbed was held in solution. As the pH rose somewhat it appears that the Fe^{+++} ions, which had entered solution under the influence of the acid, precipitated some of the phosphorus between pH 2 and 3 where

$FePO_4$ has a minimum solubility; whereas, between pH 3 and 4, Al^{+++} ions, which also had been brought into solution by the acid, exerted their maximum effect upon the phosphorus and held the solubility low. At pH 5 the precipitating effect of Al^{+++} and Fe^{+++} ions was no longer in control, and Ca^{++} ions could not yet exert their maximum effect; hence, a maximum appeared in the solubility curve. As the pH was raised by different bases the curve descended again under the influence of the Ca^{++} ions.

The foregoing explanation is based upon the work of Gaarder (4), Teakle (14), Patten (11), and Line (7), and upon the results presented in the preceding series of experiments.

The data included in tables 2, 4, and 7 show that small quantities of CaO exert a much more pronounced effect upon pH and phosphorus solubility than do large quantities of $CaCO_3$. No phosphorus, detectable by the method used, occurred in solution in these experiments at a pH above 7.46.

In no case did NaOH completely precipitate the phosphorus, even though the pH of the filtrate was raised to 8. The slight solubility and the slight degree of ionization of calcium soil together with the effect of the dissimilar Na^+ ions were probably the main cause of incomplete precipitation. The minimum amount of soluble phosphorus recovered following the application of NaOH was less than that found in the same series when $CaCO_3$ was used. This also indicates that the pH must be near 7.5 before Ca^{++} ions can exert their maximum precipitating effect upon phosphorus.

SUMMARY

In order to study the effect upon the solubility of phosphorus when the pH of a system was changed by different reagents, various compounds of calcium, or a soil saturated with calcium alone and in varying combination, were added to portions of a standardized solution of H_3PO_4.

Conclusions drawn from the results obtained may be summarized as follows:

Ca^{++} ions did not precipitate phosphorus from solution until the pH approached 5.5. Maximum precipitation or minimum solubility was not reached until the pH approached 7.5.

Large excesses of $CaCO_3$ failed to precipitate completely the phosphorus from solution, apparently because of its slight solubility.

Slight additions of CaO reduced the phosphorus in the filtrate to a minimum at an average pH of 7.36 and held it there at the higher pH values.

Large excesses of $CaCl_2$ precipitated no phosphorus from solution until the pH was raised by NaOH. Minimum solubility occurred at pH 7.36 and remained constant when the pH was extended above this value.

The soil saturated with calcium precipitated phosphorus much like a sparingly soluble calcium salt from a weak acid. The phosphorus solubility curve with this soil is similar to that with $CaCO_3$ but, probably because of adsorption, is displaced with respect to it.

When the pH of the calcium soil was varied with HCl, the added phosphorus failed, until a pH of almost 0 was reached, to remain completely in solution. A minimum appeared in the solubility curve at pH 2.56; then a gradual rise occurred to define a maximum at approximately pH 5. The minimum at pH 2.56 may be ascribed to the removal of phosphorus by Fe^{+++} ions which had entered the solution under the influence of the acid; whereas the

maximum at pH 5 may be ascribed to the fact that the Fe^{+++} and Al^{+++} ions could no longer exert their maximum precipitating effect upon the phosphorus, and the maximum precipitating effect of the Ca^{++} ions had not been attained. Additions of NaOH, CaO, and $CaCO_3$, respectively, lowered the curve from the maximum at pH 5, but to different extents and by slightly different paths. CaO caused complete precipitation of phosphorus at pH 7.46 and held it there at the higher pH values. $CaCO_3$ did not raise the pH above neutrality and did not cause complete precipitation of the phosphorus. NaOH raised the pH to 8, but complete precipitation of the phosphorus did not occur, probably because of the slight solubility and low degree of ionization of the soil.

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ESTIMATION OF AVAILABLE PHOSPHATES IN SOILS BY CO_2 EXTRACTION

AMAR NATH PURI AND A. G. ASGHAR

Irrigation Research Institute, Lahore (India)

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An extensive literature has grown round the problem of estimating available phosphates in soils. From the classical method of Dyer (3) to the recent method of Truog (6), the object has always been to simulate natural conditions. It has been admitted by practically every worker that the ideal reagent for extracting available phosphates would be a solution of CO_2. The difficulty so far has been the lack of some simple technique which could be easily reproduced and by which the results could be duplicated. It is the object of this paper to describe the development of such a technique. No claim is made as to the value of the results in predicting the behavior of the soil in the field. As a matter of fact, opinion will always be divided on that point. The proposed method is, however, recommended for trial in laboratories where better facilities exist for such tests.

The mechanism by which plants extract phosphates from soils is little understood. The most probable conjecture is that the CO_2 given out by the roots helps in dissolving the otherwise insoluble phosphates present in the soil. Most of the extraction methods are based on this conjecture. A notable departure from this idea is the alkaline extraction method of Das (2), the practical application of which has been emphasized by the work of Hockensmith et el. (4). The great importance of standardizing a method in which water saturated with CO_2 constitutes the extraction medium, lies in the fact that its application would form one of the strongest arguments for or against the CO_2 theory of root absorption.

EXPERIMENTAL

In standardizing a method of this nature, the following factors had to be taken into consideration to ensure reproducibility of results: temperature, since the solubility of CO_2 in water depends on this; time of reaction; soil-water ratio; and influence of $CaCO_3$.

The method of extraction and estimation will be described first; and the influence of the various factors will be discussed later.

Method of extraction and estimation

Extraction was done in a pyrex U-tube of the type shown in figure 1. This enabled a continuous stream of CO_2 to be passed through the soil suspension

held in the larger limb. The suspension was thus constantly agitated with a saturated solution of CO_2. An ordinary cylinder of compressed CO_2 is very suitable for the purpose and lasts for a long time.

The soil suspension after extraction for 15 minutes is filtered, and phosphate is estimated by Deniges' method as described by Chapman (1). Four cubic centimeters of a reagent containing 2.5 per cent ammonium molybdate in 10 *N* H_2SO_4 was taken in a 100-cc. measuring flask and 25 cc. of the solution under test added to it. The volume was made about 99.5 cc., 6 drops of a 2.5 per cent solution of stannous chloride in HCl was added, and the volume was made up to 100 cc. The color developed after 15 minutes was compared with 10 cc. of a standard phosphate solution, a solution of potassium dihydrogen phosphate

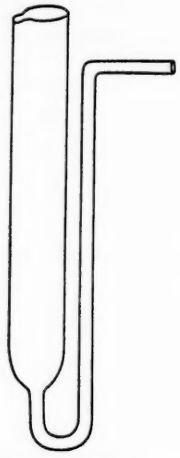

FIG. 1. DIAGRAM OF THE U-TUBE FOR THE EXTRACTION OF PHOSPHATES BY CARBON DIOXIDE

containing 1 mgm. of P_2O_5 per 100 cc., in a colorimeter. The apparatus was standardized, and its limits of accuracy were determined with pure solutions before soils were tested.

Effect of temperature

Five soils were examined in this connection. CO_2 was passed for 15 minutes at different temperatures through 2 per cent soil suspensions. The results given in table 1 show that the temperature coefficient of the reaction is not high, though the effect is more marked in some soils than in others. Maximum value, however, is obtained at low temperature in all cases. Since it is easy to maintain a temperature about 5°C. with lumps of ice, this temperature is recommended. It will be noticed, however, that results at 15°C. are not so materially different, and if it is more convenient to use that temperature, there

should be no difficulty in duplicating the results. A temperature higher than 15°C. would not be desirable.

Effect of soil-water ratio

Effect of soil-water ratio was studied on P.C. 4 soil. The results given in table 2 show that the concentration of P_2O_5 in the CO_2 extract is practically independent of the soil-water ratio, except in 1 per cent suspension, where the

TABLE 1

Effect of temperature on the value of available P_2O_5 per liter obtained by CO_2 extraction

P.C. 2 SOIL		P.C. 4 SOIL		P.C. 10 SOIL		P.C. 16 SOIL		P.C. 17 SOIL	
Temperature	P_2O_5	Temperature	P_2O_5	Temperature	P_2O_5	Temperature	P_2O_5	Temperature	P_2O_5
°C.	*mgm.*	*°C.*	*mgm.*	*°C.*	*mgm.*	*°C.*	*mgm.*	*°C.*	*mgm.*
2.0	0.4	0.6	4.26	2.0	1.04	2.0	4.02	2.0	7.9
11.6	0.25	4.0	4.16	11.6	0.60	11.6	4.00	11.6	7.2
15.0	0.24	5.6	4.18	16.0	0.57	16.2	3.96	16.4	6.28
20.0	0.24	10.0	3.94	20.0	0.66	20.0	3.24	20.0	5.80
23.0	0.19	12.4	3.94	22.5	0.45	22.4	2.08	22.2	5.70
25.0	0.24	14.6	3.76	25.0	0.55	25.0	3.68	25.0	5.60
30.0	0.27	18.4	3.62	30.0	0.55	30.0	4.74	30.0	6.04
....	....	21.8	3.62	....	....	....	....	....	....
....	....	23.6	3.57	....	....	....	....	....	....
....	....	27.3	3.46	....	....	....	....	....	....
....	....	30.4	3.47	....	....	....	....	....	....

TABLE 2

Effect of soil-water ratio on available P_2O_5 in P.C. 4 Soil

SOIL PER 100 CC.	P_2O_5 PER LITER
gm.	*mgm.*
1	3.35
2	4.54
3	4.66
4	4.96
5	5.08
7.5	4.64
10	4.64

value is slightly low. This result is very interesting and follows more logically from the conception of "availability" as the ability of the soil to supply P_2O_5. It would also appear that available P_2O_5 in a soil should not be expressed on the *weight of the soil* but in terms of the maximum concentration of the solution that can be obtained under a particular set of conditions. All results in this paper are recorded on this basis in milligrams of P_2O_5 per liter of the extracting solution. A 2 per cent suspension is recommended for routine work.

Effect of time of reaction

Effect of time of reaction was studied by passing CO_2 for varying lengths of time through a 2 per cent suspension of a soil rich in phosphates kept at 10–15°C. Results given in table 3 show that equilibrium is attained in about 10 minutes and that there is no appreciable increase when CO_2 is passed for a longer period. The effect of leaving the suspension for varying lengths of time before filtration after passing CO_2 for 15 minutes was also studied. One set was kept at 5°C. after CO_2 was passed, and the other set was kept at ordinary tempera-

TABLE 3

Effect of time of extraction on available P_2O_5

TIME	P_2O_5 PER LITER
minutes	*mgm.*
5	8.4
10	9.6
15	9.56
30	9.68
60	9.68
90	9.60
120	9.68

TABLE 4

Effect of keeping the suspension for different lengths of time after passing CO_2

TIME	TEMPERATURE WHEN FILTERED	P_2O_5 PER LITER
minutes	°C.	*mgm.*
0	3.5	9.08
10	10.2	8.64
10	3.5	8.84
15	11.8	9.48
15	3.5	8.80
30	15.0	9.48
30	3.5	8.48
60	20.0	9.12
60	3.5	8.48
90	22.1	8.92
90	3.5	8.40
120	24.3	8.80
120	3.5	8.70

ture (the temperature at the time of filtration is also recorded in this case). The results, given in table 4, show that the suspension after extraction can be kept for 2 hours at ordinary temperature without effect on the accuracy of the results. This shows that the P_2O_5 once extracted is not reprecipitated even when CO_2 is stopped. Ordinarily there would be no necessity of keeping the suspension so long after extraction, but the point was of interest as filtration takes some time, during which CO_2 may be lost. It is clear, however, that there is no likelihood of any error being introduced on that account.

Influence of $CaCO_3$

There is ample evidence in the literature to show that phosphates when added to calcareous soils are rendered unavailable. Thus Scarseth and Tidmore (5) found that yield with a high dressing of tricalcium phosphate was decreased about 60 per cent by the $CaCO_3$ when it was applied at planting, and

TABLE 5

Effect of $CaCO_3$, $CaSO_4$, or $CaCl_2$ on available P_2O_5

$CaCO_3$	P_2O_5 PER LITER	$CaSO_4$	P_2O_5 PER LITER	$CaCl_2$	P_2O_5 PER LITER
per cent	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*
0	8.4	....	....	....	....
1	1.68	0.2	7.9	0.55	6.9
2	0.70	0.4	7.12	1.10	5.92
3	0.66	0.6	7.06	1.65	5.84
4	0.50	0.8	7.5	2.2	5.76
5	0.50	10.0	1.51	....	....
6	0.45	20.0	0.72	....	....
7	0.47	30.0	0.64	....	....
8	0.41	....	....	....	....
9	0.41	....	....	....	....
10	0.41	....	....	....	....

TABLE 6

Replicate determinations of available P_2O_5 in soils

SOIL NUMBER	P_2O_5 PER LITER							
	Rubbing				Without rubbing			
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	0.26	0.28	0.26	0.26	0.24	0.26	0.28	0.28
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	2.66	3.04	3.22	2.80	2.52	2.82	2.80	2.82
6	4.60	5.52	4.80	4.60	4.64	4.60	4.70	4.60
7	0.28	0.20	0.20	0.23	0.22	0.22	0.23	0.22
8	0.93	0.92	0.96	1.00	0.93	1.04	1.03	0.98
9	3.62	3.52	3.62	3.65	3.55	3.65	3.68	3.62
10	2.14	2.50	2.58	2.70	2.72	2.63	2.60	2.60
11	0.58	0.56	0.56	0.58	0.57	0.68	0.56	0.59
12	7.02	7.24	7.24	7.21	7.36	7.40	7.20	7.30
13	7.72	8.0	7.92	7.95	7.96	8.04	7.92	8.02
Dates...	6/2/34	7/2/34	10/2/34	15/2/34	6/2/34	7/2/34	10/2/34	15/2/34

about 97 per cent by the $CaCO_3$ when it was applied 180 and 365 days before planting. These authors put forward the view that calcium in the soil solution probably united with soluble phosphorous to form tri-calcium phosphate, thus lowering the availability of the phosphorous to plants and decreasing the yield.

The question of whether the method of finding available phosphates in soil should be independent of the amount of $CaCO_3$ contained in it is important, therefore. If $CaCO_3$ decreases the availability of phosphates as measured by crop yield, obviously the laboratory method must show a decrease. This is actually the case with the CO_2 extraction method, as will be seen from table 5, which records the values for available P_2O_5 with increasing amounts of $CaCO_3$. The effect of $CaSO_4$ and $CaCl_2$ was also studied, and the results are included in table 5.

It appears that all the Ca salts have a depressing effect on available phosphates, though the influence is most marked in the case of $CaCO_3$. The importance of applying phosphatic manures as top-dressing to calcareous soils thus becomes apparent.

Reproducibility of results

It is obvious that reproducibility of results is one of the first essentials of an analytical method. To test this point, 13 soils were selected at random.

TABLE 7

Comparison of Truog method and CO_2 extraction method

SOIL NUMBER	P_2O_5 PER LITER	
	Truog's method	CO_2 extraction method
	mgm.	*mgm.*
5	6.24	8.0
6	3.4	5.24
7	3.32	3.16
9	5.0	2.84
18	1.18	0.65
27	13.68	9.28
29	1.00	1.56
41	0.5	0.38

Available phosphate was determined eight times on different days. The experiment was divided into two series: in one, the soil was thoroughly rubbed with a rubber pestle before extraction, and in the second, no preliminary treatment was given. The results recorded in table 6 leave no doubt as to the reproducibility of results. It is also seen that rubbing with a rubber pestle has no effect.

Comparison with Truog method

The CO_2 extraction method was compared with the Truog method in which the extraction is done with a buffer mixture of H_2SO_4—K_2SO_4 of pH 3. The results, given in table 7, show a general similarity, which is evidently due to the fact that extraction is effected at approximately the same pH value in both cases.

SUMMARY

A simple method for determining available phosphates in soils by CO_2 extraction has been described.

The method has been shown to give reproducible results with a number of soils. It is free from all complicated details, and the results are unaffected by alterations in the soil-water ratio, the time of extraction, etc. The addition of $CaCO_3$ to the soil lowers the value for available phosphate as determined by this method.

The method shows a general agreement with the Truog method.

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ESTIMATING EXCHANGEABLE CALCIUM AND OTHER CATIONS IN SOILS

AMAR NATH PURI

Irrigation Research Institute, Lahore, India

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I. THE SODIUM CARBONATE METHOD OF ESTIMATING EXCHANGEABLE Ca IN CALCAREOUS SOILS

Estimation of exchangeable Ca in calcareous soils has always presented peculiar difficulties on account of the solubility of calcium carbonate in the reagent used for replacement. Estimating Ca in the second liter of the leachate and thus allowing for the solubility effect (1) entail double the work. The use of alcoholic solutions brings in analytical difficulties besides the expense.

The writer has recently suggested an indirect method for such soils (5) which consists in leaching the soil with ammonium carbonate when all the bases are replaced by ammonia. The latter is determined, and from the total bases thus found, bases other than Ca which appear in the filtrate are subtracted; the difference is equivalent to exchangeable calcium.

Since ammonium carbonate can convert all the exchangeable Ca into carbonate; it was logical to suppose that sodium carbonate would behave in a similar manner. If Na_2CO_3 or K_2CO_3 could convert all the exchangeable Ca into carbonate, the decrease in the CO_2 concentration of a standard solution of Na or K carbonate would be equivalent to exchangeable Ca. The present paper deals with the application of this principle to the estimation of exchangeable Ca in calcareous soils.

Experimental

In order to standardize working conditions, it was considered necessary to perform some preliminary experiments. The results of these experiments, it was hoped, would lead to a clearer understanding of the nature of the reactions involved in the new method.

The success of the sodium carbonate method must obviously depend on the insolubility of calcium carbonate in sodium carbonate solution. In order to test this point 200-cc. portions of approximately 0.05 *N* and 0.1 *N* Na_2CO_3 solution were shaken with $CaCO_3$ in the presence of increasing amounts of NaCl. After the $CaCO_3$ was allowed to settle, 100 cc. of the clear supernatant liquid was pipetted off; excess of standard HCl was added; the liquid was boiled to expel CO_2 and back-titrated with standard alkali. The results

given in table 1 show that $CaCO_3$ is practically insoluble in Na_2CO_3 solution even in the presence of NaCl.

In order to determine whether the presence of NaCl is necessary for the complete precipitation of exchangeable Ca in the soil, 10-gm. portions of a calcareous soil (from Akola) were shaken for 2 hours with 200 cc. of 0.05 *N* Na_2CO_3 containing increasing amounts of NaCl. The suspension was then filtered and 100 cc. of the filtrate titrated with standard acid using first phenolphthalein and then methyl orange as indicator, the decrease in the concentration of CO_2 ion being taken as equivalent to exchangeable Ca. The results given in table 2 show that the value is slightly low in the absence of NaCl, but increasing amounts of the latter have very little effect.

The effect of time of shaking and contact with Na_2CO_3 solution was next studied on the same soil (from Akola). Ten-gram portions of the soil were shaken with 100 cc. of 0.1 *N* Na_2CO_3 in 0.1 *N* NaCl for varying intervals of

TABLE 1

Insolubility of $CaCO_3$ *in* Na_2CO_3 *plus* NaCl *solutions*

CONCENTRATION OF NaCl	TOTAL ALKALINITY M.E. PER 100 CC.	
	1	2
0	5.49	10.97
0.05 *N*	5.51	10.95
0.1 *N*	5.50	11.00
0.2 *N*	5.46	10.91
0.5 *N*	5.44	10.76
1.0 *N*	5.36	10.88
Without $CaCO_3$	5.46	10.87

time. Exchangeable Ca was determined by noting the decrease in the CO_2 concentration as before. The results given in table 3 show that equilibrium is established in 2 hours.

Detailed description of the proposed method

After several trials the following technique was found to be most suitable, though strict adherence to detail is not at all necessary as long as we bear in mind that the fundamental reaction involved is the precipitation of a Ca salt as $CaCO_3$, a reaction already well known in analytical chemistry:

Ten to twenty grams of soil is shaken for 2 hours with 200 cc. of 0.05 *N* Na_2CO_3 in *N* NaCl (K_2CO_3 and KCl serve equally well). The suspension is then filtered through a fluted filter, and 50 cc. of the filtrate is titrated as follows: Phenolphthalein indicator is added and standard HCl run in till the solution is colorless. The acid used is equivalent to half the carbonates in solution. A few drops of brom thymol blue indicator are then added, excess of acid is run in, and the solution is back titrated with standard alkali after boiling. The total quantity of acid added (including the phenolphthalein

titer) minus the alkali used for back titration gives the total alkalinity of the solution, i.e., the carbonate and bicarbonate. Bicarbonate is obtained by subtracting from this value twice the value for phenolphthalein titer.

The value of bicarbonate thus obtained is only approximate and is used as a guide to more accurate determination which is carried out as follows: To 100 cc. of the filtrate are added standard $BaCl_2$ and NaOH in quantities little more than the total alkalinity and bicarbonate respectively. The solution is then titrated with 0.1 *N* oxalic acid using thymolphthalein as indicator. The decrease in the concentration of NaOH added is equivalent to the bicarbonate in solution. Phenolphthalein can be used in place of thymolphthalein, but the latter gives a much sharper end point.

Since the same equivalent of bicarbonate contains twice as much CO_2 as does the carbonate, the total CO_2 in a mixture of carbonate and bicarbonate is obtained by adding the bicarbonate equivalent to total alkalinity.

TABLE 2

Exchangeable Ca *in a calcareous soil determined by* Na_2CO_3 *with and without* NaCl

CONCENTRATION OF NaCl	EXCHANGEABLE Ca, M.E. PER 100 CC.
0	44.4
0.05 *N*	48.4
0.10 *N*	48.8
0.2 *N*	50.4
0.5 *N*	49.6
1.0 *N*	49.2

TABLE 3

Effect of time of shaking on the value of exchangeable Ca

TIME OF SHAKING	EXCHANGEABLE Ca, M.E. PER 100 CC.
hours	
2	49.8
5	49.0
8	48.8
24	49.6
96	49.8

It might be pointed out that exchangeable Mg in soils is not precipitated along with Ca in this method but is brought into solution as carbonate. This obviously makes no change in the concentration of total CO_3 ions and therefore does not interfere in the Ca estimations. Incidentally, exchangeable Mg can be estimated in an aliquot of the filtrate. This point will be discussed more fully in a later publication dealing with the methods of estimating exchangeable Mg in soils.

In order to see how the Na_2CO_3 method compares with other methods, 14 soils were examined. The results given in table 4 show that there is good agreement among the different methods.

The application of well-known analytical principles to the estimation of exchangeable Ca in soils has resulted in the perfection of the ammonium oxalate method described in a previous publication (6) and the present sodium carbonate method. Apart from their utility as rapid methods of analysis, they are of great importance in so far as they support the chemical theory of soil colloids (3). The success of these methods leads one to the irrefutable conclusion that exchangeable Ca exists in chemical combination with the clay

complex, and the compound may for the present be regarded as calcium clayate, i.e., the calcium salt of clayic acid (4).

The fact that sodium carbonate in soils leads to the complete precipitation of exchangeable Ca throws an interesting side light on the cause of barrenness in alkali soils. It is not improbable that plants are unable to grow in such soils on account of calcium deficiency, no doubt brought about by the sodium carbonate which is invariably present in such soils.

It is interesting to recall in this connection the following conclusion of Joshi and Puri reached in their study on the influence of exchangeable ions in soil colloids on bacterial activity and plant growth (2): "There is no evidence

TABLE 4

Exchangeable Ca *in calcareous soils as determined by various methods*

SOIL NUMBER P. C.	pH VALUE	$CaCO_3$	EXCHANGEABLE Ca, M.E. PER 100 CC.		
			KCl method	$(NH_4)_2CO_3$ method	Na_2CO_3 method
		per cent			
1	8.41	36.05	5.1	3.95	5.3
2	8.21	4.87	49.9	52.35	55.7
4	8.55	0.82	6.25	7.05	5.4
5	8.77	1.64	6.00	6.10	5.3
7	9.58	5.62	0	1.40	1.9
8	8.41	0.58	16.7	16.90	18.3
10	8.71	1.02	19.25	19.16	23.5
11	8.77	2.32	22.25	21.45	25.9
13	8.53	1.85	49.2	53.8	55.3
16	8.74	2.24	3.5	4.10	2.9
17	8.20	0.67	7.65	7.80	7.8
19	8.40	1.17	16.80	19.50	18.1
21	8.25	3.31	8.80	10.45	6.1
24	8.59	2.02	4.40	6.35	1.0

to show that exchangeable sodium ions are directly toxic to plants. Their toxicity is most probably due to the adverse physical conditions set up by the exchangeable sodium." The statement may now be modified so that the "adverse condition" referred to therein may not be merely "physical" but most likely physico-chemical.

II. SINGLE-TREATMENT METHODS OF ESTIMATING EXCHANGEABLE Ca AND OTHER BASES IN SOILS CONTAINING CALCIUM CARBONATE OR GYPSUM

The potassium oxalate-acetate-carbonate method

The development of the potassium oxalate-acetate-carbonate method was based on the fact that soluble oxalates react with $CaCO_3$ only in a medium in which the latter is soluble and consequently the greater the solubility of $CaCO_3$, the greater is the amount that will react with oxalate in a given time. Since

$CaCO_3$ is practically insoluble in solutions of alkali carbonates, various mixtures of alkali carbonates and their corresponding chlorides and acetates were tried. A mixture of potassium chloride or acetate and K_2CO_3 appeared to be most suitable for the purpose. The effect of increasing amounts of K_2CO_3 in normal potassium chloride or acetate on the reaction between potassium oxalate and $CaCO_3$ as well as the precipitation of exchangeable Ca in the case of a black cotton soil (Akola) is shown in table 5.

One hundred cubic centimeters of solutions of 0.1*N* K oxalate in *N* KCl or *N* K acetate with increasing amounts of K_2CO_3 were shaken with 2 gm. of $CaCO_3$ in one series and with 10 gm. of soil in the other. After being shaken intermittently by hand for half an hour, the mixture was filtered through a dry fluted filter. Fifty cubic centimeters of the filtrate was titrated

TABLE 5

Removal of oxalate ion by $CaCO_3$ and Akola soil in the presence of increasing amounts of K_2CO_3

0.1 *N* K_2CO_3 IN 100 CC. SOLUTION	OXALATE ION REMOVED (AMOUNT OF 0.1 *N* SOLUTION)			
	N KCl		*N* K acetate	
	$CaCO_3$	Soil	$CaCO_3$	Soil
cc.	*cc.*	*cc.*	*cc.*	*cc.*
0	....	....	5.8	59.3
5	5.6	59.0	2.9	56.7
10	1.8	55.4	1.3	52.4
15	0.6	52.6	0.6	52.4
20	0.4	50.8	0.6	51.4
25	0.2	49.2	0.5	50.0
30	0.4	49.0	0.6	49.4
40	0.25	46.6	...	....
50	0.2	45.6	...	....
75	0.1	42.4	...	....
100	0	39.8	...	....

with standard $KMnO_4$ and decrease in the concentration of oxalate noted. This decrease is equivalent to the $CaCO_3$ that reacted with K oxalate in the one case and with the exchangeable Ca in the other. It is seen that above a certain concentration of K_2CO_3 there is practically no reaction between K oxalate and $CaCO_3$; but exchangeable Ca, as determined by the disappearance of the oxalate ion, continues to decrease. What actually happens is that when the concentration of K_2CO_3 is small some K oxalate reacts with $CaCO_3$ in the soil, giving too high a value for exchangeable Ca. At the point where the reaction between K oxalate and $CaCO_3$ ceases, the decrease in oxalate ion is equivalent to exchangeable Ca. When the amount of K_2CO_3 is increased still further, there is a competition between K oxalate and carbonate to precipitate Ca as oxalate or carbonate. Since increasing amounts of K_2CO_3 would lead to the formation of increasing amounts of $CaCO_3$, the value for

exchangeable Ca decreases continuously. The logical conclusion is that the concentration of K_2CO_3 should be kept within certain limits so as to render $CaCO_3$ insoluble but not to induce the precipitation of exchangeable Ca as carbonate.

Other experiments showed that K acetate is superior to KCl in the oxalate-carbonate mixture, as the latter gives slightly low values in the case of non-calcareous soils. The use of K oxalate-acetate-carbonate mixture ensures one uniform procedure for calcareous as well as carbonate-free soils.

The most suitable medium having been found for the precipitation of Ca oxalate in the presence of $CaCO_3$, an experiment was performed to determine whether the amount of $CaCO_3$ had any effect on its reaction with K oxalate. It was found that when the amount of $CaCO_3$ was increased from 1 to 10 gm., the portion that reacted with K oxalate remained practically constant. It was concluded, therefore, that the percentage of $CaCO_3$ in a soil will have no influence on the determination of its exchangeable Ca by this method.

The effect of temperature on the rate of reaction was next studied. It was found that higher temperatures slightly favored carbonate formation, that a temperature below 20°C. was most favorable to the exclusive formation of Ca oxalate, and that a half hour's shaking by the hand was quite sufficient for the completion of the reaction. In tropical countries like India where the temperature in summer is high, an ordinary galvanized iron tank containing water cooled to 15°C. with ice may be used. No temperature control is required; it is only necessary to avoid too high a temperature.

Summary description of the method. Ten grams of soil is shaken in a stoppered bottle by the hand or in an end over end shaker for about half an hour (in the case of heavy soils the shaking is continued for 1 hour) with 100 cc. of a solution *N* with respect to K acetate, 0.1 *N* with respect to K oxalate, and 0.015 *N* with respect to K_2CO_3, filtered, and 50 cc. of the filtrate titrated with 0.1 *N* $KMnO_4$. The total decrease in the concentration of oxalate ion is equivalent to exchangeable Ca in the given weight of the soil. If the room temperature is high the suspension should be cooled to 15°C.

The ammonium oxalate-acetate-carbonate method

It was found in the preliminary experiments that when ammonium salts were substituted for potassium salts $CaCO_3$ reacted with the oxalate ion to a large extent. It appeared, however, that, though the analogy broke down when equivalent concentrations were used, there might be a different proportion of the oxalate-acetate-carbonate mixture in the case of ammonium salts in which $CaCO_3$ might be insoluble. The additional advantage of such a mixture would be that ammonium salts could be removed, leaving behind all the other exchangeable bases as carbonates, and therefore the method could be used for determining other cations.

In the application of the ammonium oxalate method to calcareous soils three important factors were determined: the proportion of ammonium car-

bonate that would render $CaCO_3$ insoluble but would not precipitate exchangeable Ca as carbonate—0.25 *N*; the optimum temperature at which the reaction should be allowed to take place—about 10°C.; and the time of shaking the soil with the reaction mixture—half an hour.

Summary description of the method. Twenty grams of soil is added to 200 cc. of a solution 0.1 *N* with respect to ammonium oxalate, 0.5 *N* with respect to ammonium acetate, and 0.25 *N* with respect to ammonium carbonate, cooled to 10°C.

The Ca is determined as in the K oxalate-acetate-carbonate method. For the other cations 100 cc. of the filtrate is evaporated to dryness and gently ignited, care being taken to avoid spurting. The residue, which consists of the carbonates of exchangeable Na, K, and Mg, is taken up with the least amount of boiled water and filtered, followed by one or two leachings. The combined filtrate consists of exchangeable Na and K; whereas the residue on the filter paper is exchangeable Mg as carbonate. Both are titrated separately with standard acid, directly in the case of Na and K, residually in the case of Mg.

Soils rich in organic matter sometimes yield a filtrate which is highly colored and therefore cannot be titrated directly with permanganate. For such soils the following modification is suggested: Fifty cubic centimeters of the filtrate is acidified with acetic acid, and the oxalic acid is precipitated by adding excess $CaCl_2$ to the hot solution. Since the maximum amount of oxalic acid present is known, a great excess can be easily avoided. The precipitate of calcium oxalate is filtered, washed, and ignited to convert it into carbonate or oxide, which is then titrated residually after excess standard acid has been added. This titer is equivalent to the oxalate present in the solution, from which the decrease in the concentration of oxalate ion is computed. Other bases are, of course, determined as usual by igniting an aliquot and titrating the residue.

Comparison with other methods

The following methods were tried:

(*A*) *NaCl method.*—Ten grams of soil is leached with 1000 cc., of 0.2 *N* NaCl in 100-cc. lots, followed by another 1000-cc. leaching in the same way. Ca is determined in the two leachates separately, and the difference between the two is taken as equivalent to exchangeable Ca.

(*B*) *KCl method.* Exactly like (*A*) but 0.2 *N* KCl is used instead of NaCl.

(*C*) *Ammonium acetate method.* Ten grams of soil is leached with 1 l. of *N* ammonium acetate in 100-cc. lots; Ca is determined in an aliquot of the leachate. This method is used for carbonate-free soils only.

(*D*) *NH_4Cl method.* Ten grams of soil is leached with 1 l. of *N* NH_4Cl in 100-cc. lots; Ca is determined in the leachate. This method is used for carbonate-free soils only.

(*E*) *$(NH_4)_2CO_3$ method.* Ten grams of soil is leached with 500 cc., of *N*

$(NH_4)_2CO_3$. Total ammonia taken up by the soil minus the exchangeable Na, K, and Mg in the leachate is taken as equivalent to exchangeable Ca (5).

(*F*) *Na_2CO_3-NaCl method.* Ten grams of soil is shaken for 2 hours with 200 cc. of 0.1 *N* Na_2CO_3 solution in *N* NaCl. An aliquot of the filtrate is

TABLE 6

Exchangeable Ca in calcarious soils by various methods

SOIL NUMBER	$CaCO_3$	pH	CLAY	EXCHANGEABLE Ca, per 100 GM. SOIL								Na PLUS K	Mg
				(A)	(B)	(E)	(F)	(G)	(H)	(I)	(J)		
	per cent		*per cent*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*
1	36.05	8.41	11.3	5.4	5.1	3.95	5.3	4.8	7.6	4.1	8.4	0.4	1.7
2	4.87	8.21	59.3	47.9	49.9	52.35	55.7	49.6	46.2	48.0	48.0	2.3	10.0
4	0.82	8.55	15.2	9.15	6.25	7.05	5.4	8.4	9.0	7.4	8.4	0.4	0.6
5	1.64	8.77	12.3	7.00	6.00	6.10	5.3	8.4	9.1	6.6	8.6	0.2	1.0
7	5.62	9.58	21.8	3.00	0	1.4	1.9	3.4	3.9	2.2	2.0	9.2	5.8
8	0.58	8.41	25.2	18.35	16.7	16.9	18.3	19.8	21.2	19.0	19.0	1.0	2.0
10	1.02	8.71	35.6	20.75	19.25	19.16	23.5	23.2	25.5	22.8	22.0	1.5	3.3
11	2.32	8.77	32.8	23.1	22.25	21.45	25.9	27.6	28.1	26.0	28.4	0.8	4.6
13	1.85	8.53	58.9	48.3	49.2	53.8	55.3	54.8	55.6	50.6	51.0	0.4	5.5
16	2.24	8.74	7.3	3.6	3.5	4.1	2.9	3.6	5.8	4.4	5.8	0.25	2.9
17	0.67	8.20	14.2	9.0	7.65	7.8	7.8	10.4	8.6	7.2	....	...	...
19	1.17	8.40	42.4	15.4	16.8	19.5	18.1	21.0	19.0	17.2	18.0	0.5	3.0
21	3.31	8.25	13.5	7.05	8.8	10.45	6.1	11.4	12.0	9.0	....	...	...

TABLE 7

Exchangeable Ca in carbonate-free soils by various methods

SOIL NUMBER	pH	CLAY	EXCHANGEABLE Ca, PER 100 GM. SOIL							Na PLUS K	Mg
			(C)	(D)	(F)	(H)	(I)	(J)	(K)		
		per cent	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*
3	7.64	62.3	53.8	50.8	53.2	55.8	53.4	51.4	59.6	1.1	12.4
6	5.29	28.4	5.1	4.9	0.6	6.3	3.8	4.2	5.4	0.3	1.5
9	5.76	21.6	3.8	4.45	0.6	4.2	1.3	2.2	4.6	0.9	0.8
12	5.84	3.8	3.6	3.95	0	4.4	0	2.2	3.2	0.7	1.6
14	5.37	22.3	2.8	3.75	5.7	5.6	6.0	5.4	5.0	0.15	0.65
15	7.71	21.9	13.0	13.9	12.0	12.0	8.6	8.6	12.0	0.1	0.9
18	5.79	22.2	4.1	4.3	4.5	4.0	2.2	...	3.6	...	...
20	5.64	6.5	3.1	4.8	4.5	2.3	...	0.2	2.2	0.15	1.2
22	6.85	15.2	7.85	8.1	8.2	8.5	...	...	8.5	...	...
23	7.41	11.3	8.2	9.05	5.6	8.4	6.5	...	9.0	...	...

titrated with standard acid using phenolphthalein first and then methyl orange as indicator. The decrease in the concentration of CO_3 ion is taken as equivalent to exchangeable Ca (see Part I).

(*G*) *K_2CO_3-KCl method.* Exactly like (*F*) but K salts are used instead of Na salts.

(*H*) *K oxalate-acetate-carbonate method.* Method exactly as described in this paper.

(*I*) *K oxalate-chloride-carbonate method.* Exactly like (*H*) except that KCl is used in place of K acetate.

(*J*) *Ammonium oxalate-acetate-carbonate method.* Exactly as described in this paper.

(*K*) *Ammonium oxalate-acetate method.* Exactly like (*J*) but without $(NH_4)_2CO_3$. This method is used for carbonate-free soils only.

The results of this comparison are given in tables 6 and 7. Exchangeable Na + K, and Mg in the soils were determined by the ammonium oxalate-acetate-carbonate method. The various methods show a good agreement when the nature of a material like soil and the inaccuracies of the leaching methods are considered.

The general agreement among the various methods also disposes of the question of the fate of oxalate in soils. It is quite clear that we need not have recourse to absorption phenomenon to explain its disappearance. One or two highly acid soils were encountered which yielded with the oxalate-acetate-carbonate mixture an extract which was capable of being oxidized by permanganate solution, thus leading to low values for exchangeable Ca. The amount thus brought out was never more than 1 to 2 m.e. per 100 gm. of soil, however, and could always be allowed for by running a blank.

Application of the single-treatment methods to soils containing gypsum

The methods discussed in the foregoing, obviously, are not applicable to soils containing gypsum, which must be removed or rendered insoluble before they are applied. A technique was developed by which all the $CaSO_4$ is converted into $CaCO_3$. Briefly, the method consists in shaking the soil suspension with excess $BaCO_3$ when the following reaction takes place:

$$CaSO_4 + BaCO_3 \rightarrow CaCO_3 + BaSO_4$$

The excess $BaCO_3$, as well as the products of reaction, is insoluble in the medium in which the final precipitation of exchangeable Ca is effected.

For standardizing experimental conditions, gypsum was added in known amounts to a black cotton soil from Akola, since soils containing varying quantities of gypsum were not available. The effectiveness of the method of removing $CaSO_4$ was judged by the nearness of the value of exchangeable Ca in the treated soil to its true value in untreated Akola soil as previously determined by various methods.

Speed of reaction between $CaSO_3$ and $BaCO_3$. On account of the limited solubility of both $CaSO_4$ and $BaCO_3$, it was necessary to study the time required for the complete precipitation of $CaSO_4$. To 10-gm. portions of Akola soil were added 0.5 gm. of $CaSO_4$ and 1 gm. of $BaCO_3$. The mixture was shaken with 50 cc. of water for varying lengths of time, and exchangeable Ca was determined as described in this paper. Controls were run with no soil.

It was assumed that when the reaction between $CaSO_4$ and $BaCO_3$ was complete, the soil would give the original value for exchangeable Ca. The results given in table 8 show that in the case of soil and gypsum 2 hours' shaking is quite sufficient to bring about the complete precipitation of $CaSO_4$ so that practically the same value for exchangeable Ca is obtained as without gypsum. It is, of course, possible that in natural soils gypsum may not be in such a soluble state and longer shaking may be required, but this could be easily ascertained by individual workers for the soils they might be dealing with. In any case, overnight shaking is recommended in the standard technique to insure completion of the reaction.

It might be noted that when dilute H_2SO_4 is added to the filtrate prior to titration with $KMnO_4$, a white precipitate of $BaSO_4$ is noticed. This is due

TABLE 8

Speed of reaction between $CaSO_4$ and $BaCO_3$

MIXTURE	TIME OF SHAKING	OXALATE REMOVED
	hrs.	*m.e.*
$CaSO_4 + BaCO_3$	0	5.18
	0.5	0.89
	1	0.49
	2	0.26
$CaSO_4 + BaCO_3$ + soil	2	5.23
	5	5.30
	10	5.40
	20	5.38
Soil alone	..	5.24
$CaSO_4$ alone	..	5.45
$BaCO_3$ alone	..	0

to the presence of some barium oxalate in the solution and does not interfere with the titrations or the accuracy of the method.

Next, the effect of increasing amounts of $CaSO_4$ in Akola soil on the estimation of exchangeable Ca in it was tried: 0.5 to 2 gm. of $CaSO_4$ were added to 10 gm. of soil. This corresponded with 5 to 20 per cent of gypsum. The amount of $BaCO_3$ was increased proportionately and was kept at double the amount of $CaSO_4$ per 10 gm. of soil. The results given in table 9 show that the amount of exchangeable Ca is practically unaffected by the presence of $CaSO_4$, provided the soil is first shaken with $BaCO_3$.

In order to determine whether the principle just enunciated was applicable to other types of soils, a number of calcareous soils were examined for their exchangeable Ca, before and after the addition of 5 per cent gypsum and shaking with $BaCO_3$. The results given in table 10 show that in most cases there is a slight increase in the exchangeable Ca after the addition of gypsum.

This is evidently because the soils that were not in equilibrium with $CaSO_4$ took some more calcium in the exchangeable form, resulting in a higher value. It is important to note, however, that this slight increase (the highest being 0.6 m.e.) could not account for the whole of the $CaSO_4$ (5.8 m.e.) added to the soil.

Detailed description of the proposed method. Ten grams of soil is shaken overnight with 50 cc. of water and 1 gm. of $BaCO_3$ in a stoppered bottle, cooled to 15°C., and 50 cc. of the potassium or ammonium oxalate-acetate-

TABLE 9

Exchangeable Ca in Akola soil with increasing amounts of $CaSO_4$ in 10 gm. of soil

GYPSUM ADDED	$BaCO_3$ ADDED	EXCHANGEABLE Ca
gm.	*gm.*	*m.e.*
0	0	5.62
0.5	1	5.64
1.0	2	5.74
1.5	3	5.82
2.0	4	5.90

TABLE 10

Exchangeable Ca in soils with and without gypsum

SOIL NUMBER	CLAY	pH	EXCHANGEABLE Ca PER 100 GM. SOIL	
			With gypsum	Without gypsum
	per cent		*m.e.*	*m.e.*
1	11.3	8.41	10.4	9.6
4	15.2	8.55	15.8	9.0
5	12.3	8.77	14.6	9.1
7	21.8	9.58	3.0	3.9
8	25.2	8.41	25.8	21.2
10	35.6	8.71	31.8	25.5
11	32.8	8.77	33.0	30.0
13	58.9	8.53	56.6	55.6
16	7.3	8.74	11.4	5.8
17	14.2	8.20	14.4	8.6
21	13.5	8.25	14.3	12.1

carbonate mixture is added. The procedure followed is the same as for the K oxalate-acetate-carbonate method.

If the soil contains sulfates of alkali metals besides $CaSO_4$, these must be removed by leaching with alcohol (rectified spirit), before the soil is shaken with $BaCO_3$. The reason for this precaution is that alkali sulfates can react with $BaCO_3$ to give $BaSO_4$ and alkali carbonates, and the latter can react with exchangeable Ca and remove it as carbonate (6); therefore, at the end of the first reaction some of the exchangeable Ca might be precipitated along with the

$CaSO_4$. Leaching with alcohol leaves the soil in a friable state on drying, and it can be detached easily from the filter paper for the determination of exchangeable Ca.

The influence of alkali sulfates will be readily understood from the following experiment in which 10-gm. portions of Akola soil were shaken with 0.5 gm. of $CaSO_4$ and 1 gm. of $BaCO_3$ in the presence of increasing amounts of sodium sulfate. The results given in table 11 show that the amount of exchangeable Ca decreases as the sodium sulfate is increased in the soil. The decrease in

TABLE 11

Effect of Na_2SO_4 on exchangeable Ca in Akola soil in the presence of 5 per cent gypsum

Na_2SO_4 PER 100 GM. SOIL	EXCHANGEABLE Ca PER 100 GM. SOIL AFTER $BaCO_3$ TREATMENT
m.e.	*m.e.*
0	52.4
10	44.4
20	36.4
30	31.0
40	29.2
50	27.0

TABLE 12

Various forms of Ca in Sind soils

SOIL NUMBER	SOLUBLE SALTS	Ca PER 100 GM. SOIL IN VARIOUS FORMS		
		Exchangeable	$CaSO_4$	$CaCl_2$
	per cent			
1	18.2	3.2	5.2	28.4
3	15.76	5.2	2.4	29.8
4	29.18	4.6	30.6	19.6
7	23.02	4.8	23.6	25.8
8	10.74	0	15.6	3.6
9	2.24	7.0	9.4	7.0
10	57.4	2.2	32.0	7.0
12	13.28	6.4	2.8	30.0

exchangeable Ca, however, is not proportional to the Na_2SO_4 present; in fact, after the first 3 m.e., the effect is very much reduced.

As an example of the practical application of the results of this investigation, the analyses are recorded of certain soils from Sind which contained $CaCl_2$, $CaSO_4$, and Na_2SO_4. Incidently the technique developed herein enables us to differentiate between the various forms of Ca. The following determinations were made: (A). The soil was leached with 200 cc. of alcohol, and the filtrate was examined for $CaCl_2$ and alkali sulfates. (B). The alcohol-leached soil was shaken with 50 cc. of water and 1 gm. of $BaCO_3$ and then

examined for exchangeable Ca. (C). The alcohol-leached soil was examined for exchangeable Ca without being shaken with $BaCO_3$. (A) gave $CaCl_2$, (C)—(B) gave $CaSO_4$, and (B) gave exchangeable Ca.

The results given in table 12 bring out clearly the characteristic difference in the soils. It will be seen that a number of soils, although containing a large amount of water-soluble Ca, have very little exchangeable Ca. This is, of course, due to a very large excess of sodium salts.

SUMMARY

A new method of estimating exchangeable Ca in calcareous soils has been described. It consists in shaking the soil with 0.05 *N* Na_2CO_3 in *N* NaCl. The decrease in the concentration of CO_2 ions is equivalent to exchangeable Ca in the soil.

The importance of the method in supporting the chemical theory of soil colloids and its bearing on the cause of barrenness in alkali soils are pointed out.

Exchangeable Ca in calcareous soils can also be determined by shaking a known weight of the soil with a definite mixture of potassium or ammonium oxalate-acetate-carbonate. In this mixture $CaCO_3$ is insoluble, and the decrease in the concentration of oxalate ion is equivalent to exchangeable Ca.

Soils containing gypsum require a preliminary treatment with excess $BaCO_3$ when the $CaSO_4$ is converted into $BaSO_4$ and $CaCO_3$, and thus rendered insoluble.

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ROOT NODULE BACTERIA OF SOME TROPICAL LEGUMINOUS PLANTS: I. CROSS-INOCULATION STUDIES WITH *VIGNA SINENSIS* L.[1]

O. N. ALLEN AND ETHEL K. ALLEN

University of Hawaii

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The contributions of Lewis and Nicholson (9), Maassen and Müller (11, 12), Simon (18), Garman and Didlake (5), Burrill and Hansen (2), and others have established the classification of leguminous plants into cross-inoculation groups with respect to the root nodule-forming bacteria. In the last two decades so much information has accumulated concerning this relationship between the host plant and its nitrogen-fixing symbiont that at present about 20 recognized groups exist.

Of those plants which have been placed in cross-inoculation groups the majority are forage or cover crops common to temperate regions, although many have their origin in tropical and subtropical areas. Relatively few large shrubs and trees have been investigated. The present study has had a two-fold objective: (*a*) a survey of the nodule formation on the root systems of some of the more readily accessible leguminous plants growing in the Territory of Hawaii, and (*b*) the isolation of Rhizobia from the nodules of tropical species to be used as inocula in plant tests. The data reported, accumulated over a period of 5 years, concern various leguminous vines, shrubs, and trees found only in the botanical gardens and arboreta on the island of Oahu, and other leguminous plants more or less common throughout the Territory.

The Territory of Hawaii has an extensive leguminous flora. Although Rock (15) listed only 70 genera and 200 species of leguminous plants for the Territory of Hawaii in 1918, the number of species, because of the careful and extensive introductions made by various societies and experiment stations, has probably doubled in the past 18 years. For most of these introductions credit is due the Experiment Station of the Hawaiian Sugar Planters' Association. Practically all of the leguminous plants concerned in this study were introduced into Hawaii from other tropical areas, since only 8 of the 22 indigenous species listed by Rock (15) are mentioned.

EXPERIMENTAL

The scientific name of each plant studied was regarded of primary importance, the common name as secondary. In those instances where the authors

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were in doubt as to the true scientific name of the plant, plant parts were brought into the laboratory and taxonomic keys consulted. Frequently taxonomic experts accompanied the authors on the various field trips and classified the plants *in situ*. Ultimately the scientific names of all the plants herein reported were checked and verified by competent taxonomists of island flora.[2]

Various treatises on tropical flora, in addition to *Index Kewensis*, have constituted the background for the plant classifications. Rock's monographs on the *Leguminous Plants of Hawaii* and the *Indigenous Trees of the Hawaiian Islands* were especially valuable. For the naming of the plants more recently introduced, the contributions of Merrill (13), Ridley (14), Britton and Wilson (1), and others were consulted.

DISTRIBUTION AND DESCRIPTION OF NODULES

Root systems of all the plants listed in table 1 were examined during the growth of the plants in forest or field soil. Although, because of the variables naturally encountered, no attempts were made to determine quantitatively the number of nodules per plant, an effort was made to ascertain the relative extent of nodulation. In the cases of the trees this aim was accomplished by a careful removal of the soil from a large portion of the root mass. On a few occasions when this procedure was thought inadequate, a more extensive examination was made by removing practically the entire root system of the species from the soil. Root systems of the vines, herbs, and many of the shrubs such as the species of Indigofera, Crotalaria, and Alysicarpus, were, of course, removed from the soil without difficulty. When the soil was too rocky, hard, or sticky to permit a satisfactory examination of the root systems, seeds were collected and subsequently planted in various lots of island soil, and the resulting plants were examined during periods of growth.

There seemed to be no correlation between the age or size of any of the plants and the extent of nodulation. This was of particular interest, since the ages of the trees examined varied from 1 to 20 years and their heights from 5 to 40 feet. Considerable variations occurred in the sizes, numbers, and distribution of nodules on any one species of the trees examined. As a rule, the young nodules were round, white, and smooth-surfaced, whereas the older forms assumed variously branched shapes, were commonly black or dark brown and rough-surfaced, and tended to be more or less of a hard "corky" or "woody" texture. Nodules on the species of Erythrina and Lonchocarpus were usually more or less centrally located on the upper root systems immediately around

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TABLE 1

Additions to the cowpea group on the basis of reciprocal cross-inoculation with Vigna sinensis *L.*

Sub-family MIMOSOIDEAE

Enterolobium cyclocarpum Griseb.
Samanea saman (Jacq.) Merrill
**Albizzia lebbek* (L.) Benth.
Albizzia retusa Benth.
Albizzia chinensis (Osbeck) Merrill
Albizzia saponaria (Lour.) Blume
Albizzia moluccana Miq.
Albizzia odoratissima Benth.
Albizzia acle (Blanco) Merrill
Albizzia lebbekoides (DC.) Benth.
Albizzia procera (Roxb.) Benth.
Albizzia katangensis Wildem.
**Acacia koa* A. Gray
Acacia arabica Willd.
Acacia koaia Hillebr.
Acacia confusa Merrill
Acacia horrida (L.) Willd.
Acacia catechu Willd.
Acacia scorpioides A. Cheval
Acacia macrantha H. and B.
Acacia spadicigera Cham. and Schlecht
Acacia robusta Burch.
Acacia richii A. Gray
Acacia auriculaeformis A. Cunn.
Acacia baileyana F. v. M.
Acacia decurrens Willd.
**Acacia farnesiana* (L.) Willd.
Acacia glaucescens Willd.
Acacia verticillata Willd.
Inga edulis Mart.
Inga laurina (Sw.) Willd.
Parkia africana R. Br.
**Parkia biglandulosa* Wight and Arn.
Parkia javanica (Lam.) Merrill

Sub-family CAESALPINIOIDEAE

**Cassia mimosoides* L.
Cassia pilifera Vog.
Hymenaea courbaril L.

Sub-family PAPILIONOIDEAE

Crotalaria assamica Benth.
Crotalaria incana L.
Crotalaria sericea Retz
Crotalaria usaramoensis Baker
Crotalaria anagyroides H.B. and K.
Desmodium uncinatum (Jacq.) DC.
Desmodium discolor Vogel
Desmodium barbatum (L.) Benth. and Oerst
Desmodium pabulare Hoehne
Desmodium cajanifolium DC.
Indigofera suffruticosa Mill.
Indigofera hirsuta L.
**Erythrina indica* Lam.
Erythrina poeppigiana Cook
Erythrina rubrinervia H.B. and K.
Erythrina arborea Small
Erythrina micropteryx Poepp.
Erythrina abyssinica Lam.
Erythrina cristagalli L.
Erythrina berteroana Urban
Erythrina velutina Willd.
Erythrina corallodendron L.
Erythrina monosperma Gaud.
Erythrina insignis Tod.
Erythrina fusca Lour.
Erythrina subumbrans (Hassk.) Merrill
Erythrina horrida Moc. and Sesse
Tephrosia candida DC.
Tephrosia purpurea (L.) Pers.
Tephrosia noctiflora Bojer
Andira inermis H.B. and K.
Piscidia erythrina L.
Stylosanthes guianensis Sw.
Vigna marina (Burm.) Merrill
Vigna owahuensis Vog.
Vigna sandwicensis A. Gray
Phaseolus lathyroides L.
Abrus precatorius L.
Alysicarpus vaginalis (L.) DC.
Alysicarpus longifolius W. and Arn.
Alysicarpus rugosus DC.
Canavalia campylocarpa Piper
Mucuna gigantea (Willd.) DC.
**Pterocarpus indicus* Willd.
Pterocarpus vidalianus Rolfe
Pterocarpus echinatus Pers.
Pterocarpus marsupium Roxb.
**Lonchocarpus sericeus* (Poir.) H.B. and K.
Lonchocarpus violaceous (Jacq.) H.B. and K.
Lonchocarpus domingensis (Pers.) DC.
Lonchocarpus latifolius (Willd.) H.B. and K.
Pongamia pinnata (L.) Merrill
Derris microphylla (Miq.) Val.
Clitoria ternatea L.

* Seeds of this species were used to test the nodule-forming abilities of the Rhizobia from other plants in this genus.

the tap root, whereas with the other species of trees nodule distribution tended to be more common on the lateral root mass.

With the exception of *Acacia baileyana* F. v. M. in the Mimosoideae and *Hymenaea courbaril* L. in the Caesalpinioideae, all of the plants listed in table 1 were moderately well nodulated. The species of Acacia, Inga, and Pterocarpus were the most heavily nodulated of the trees. Huge clusters of small and large nodules were generally distributed over the entire root systems. Leonard (8) has reported the rather general nodulation of Acacia species growing in greenhouse soil. Of 24 species (list not given) examined by him in 1924, only one, *Acacia baileyana* F. v. M., was found to lack nodules. Incidentally this species was, according to our observations, only sparingly inoculated. Several trees, each over 5 years old, were examined before nodules were found, and the positive cases noted showed only a few scattered, small nodules on very young tender roots. This was more or less unexpected, since species of Acacia are well distributed over the island, and, judged from the profuse nodulation on the other Acacia species, the root nodule organisms are equally so. On the other hand, nodulation on *Acacia decurrens* Willd. and *Acacia glaucescens* Willd. in the same general locality was exceptionally heavy.

Only one plant of *Andira inermis* H. B. & K. was available for examination; typical nodules, such as those to be noted in plate 1, figure 6, were abundant.

Nodulation of several species was of interest because of the nature and growth habits of the host plants. Nodules, averaging 10–20 mm. in length, were common on all the plants of *Piscidia erythrina* L. examined despite the fact that the root system of this species possesses Piscidin, an alkaloid ($C_{29}H_{24}O_8$) commonly used by natives in tropical areas to poison and stupefy fish. The roots were always accompanied by a most unpleasant and pungent odor. Likewise the root systems of various plants of *Derris microphylla* (Miq.) Val. were heavily nodulated. Various species of this genus are used for the extraction of rotenone.

Several variations in nodule types consistently appeared upon the root systems of species within a single genus. For instance, *Vigna marina* (Burm.) Merrill, a prostrate or climbing herbaceous annual vine common along the shore line, was always profusely nodulated despite the fact that the plants at high tide received heavy salt spray and (or) wetting by waves, and at low tide subsisted in dry sand. The nodules on this species were round, averaging 4–6 mm. in diameter, dull gray with smooth surfaces and with inky black centers. *Vigna owahuensis* Vog., a slender hispid indigenous vine common in the arid upland regions of the island, bore nodules practically identical with those commonly found on a well-inoculated clover root system. In like manner, the nodules on *Alysicarpus longifolia* W. and Arn. were tender and variously branched, similar to those on Crotalaria species, whereas those on *Alysicarpus vaginalis* (L.) DC. were round with rugged surfaces as is the type common on roots of *Vigna sinensis* L.

CROSS-INOCULATION STUDIES

Methods

The methods and techniques employed in the plant tests are given in the following detailed descriptions, since the infallibility of the results depends unquestionably upon the manner in which they were obtained.

Isolation of Rhizobia. At least five nodules were taken from the root systems of each species listed in table 1. Each nodule was washed under running tap water to remove the adhering soil, rinsed in three changes of sterile distilled water, and subjected to a violent agitation for 4–5 minutes in bichloride of mercury (1:1000). The nodule was then rinsed again in several changes of sterile distilled water, to remove the bichloride of mercury, and crushed in a tube containing 10 cc. of sterile water. Serial loop dilutions of this nodule suspension were then plated. In exceptional cases where the nodules were moderately large, a center portion of the nodule was removed for plating purposes with a sterilized needle.

Especial care was taken to avoid any probability of error due to mixed cultures or platings. Nodules of only one species were plated at one time, and, moreover, each nodule was considered a specific entity. Plates were labeled with the scientific name of the plant as well as with the index number of the species. This general procedure was followed throughout the series of isolations and pot experiments. Fresh mercuric chloride and rinse water in separate sterile dishes were used for each nodule, and, as a rule, all material pertaining to the previously plated nodule was removed from the laboratory table before the plating of another nodule was begun. In only a very few exceptional cases were nodules from more than two different species plated in one day.

Prior to Carroll's paper (3) Bacto yeast extract mannitol agar was used exclusively in the plating procedures. In the technique following this report asparagus extract mannitol agar was used in addition. On the whole this latter medium seemed preferable for many of the Rhizobia cultures, as the growth resulting from the nodule suspensions usually appeared in a shorter time. In such instances colonies suitable for isolation were usually apparent on the latter medium after 10 days' incubation at 25°C., whereas on the yeast extract mannitol agar plates suitable colonies were not apparent until 3–4 days later.

The incubation period of the cultures from the nodule suspensions varied considerably. The shortest time was required by the Rhizobia from the species of Erythrina. These colonies were usually discernible in 3–4 days and were large enough to warrant isolation in about 7 days; whereas the colonies from the nodules of the three species of Alysicarpus and from *Mucuna gigantea* (Willd.) DC. were not visible until about the fourteenth day and were not large enough to be isolated in less than 3 weeks.

At the time of isolation the colonies were examined with a Greenough-Leitz binocular microscope (6 X oculars), and only well-isolated colonies were picked

into yeast water mannitol broth. Growth as evidenced by turbidity was usually apparent after 7 days' incubation at 25°C. Replating and re-isolation of the cultures were repeated at least three times, and colonies were picked again in the manner described. The purity of the cultures was established by their reactions in litmus milk and by the characteristics of their growth on potato slants, on congo red and brom thymol blue yeast extract mannitol agars, and on asparagus extract mannitol agar. The cultural and physiological characteristics of these cultures will be reported at a later date.

Plant Tests. Verification of the isolated cultures as *bona fide* root nodule bacteria was achieved by rigidly controlled plant inoculation tests. Such tests were conducted in a special compartment of a greenhouse built in 1930. Only two sides of this compartment had outside exposure, although all sides had glass windows. The windows on the exposed side of the compartment were attached at the top by hinges and opened outward from the bottom on supporting rods. All air coming through these windows was filtered through a thin layer of cotton between two thickness of cheesecloth sewed to an inside window screen. The floor of this compartment was kept wet at all times, and the one door to this enclosure was closed except when in actual use as an entrance or exit.

The nodule-forming abilities of the newly isolated cultures were initially tested by inoculating either (*a*) seeds of their respective host plants or (*b*) seeds of a closely related species of the same genus. The latter method was used in many cases where it was not possible to obtain seeds from each of the respective species. Such examples have been designated by an asterisk in table 1. In several instances, such as with *Hymenaea courbaril* L. in the Caesalpinioideae and with *Andira inermis* H. B. & K. in the Papilionoideae, neither of the above procedures was possible, because viable seeds of these species could not be obtained locally or elsewhere. A slightly different obstacle was experienced with the seeds of the species in the genus Inga. Although a few seeds were obtained, all efforts to treat them satisfactorily were futile. In this genus the coats of the seeds resemble a soft wool-like mass from which the naked embryos escape and fall to the ground when the pods open. Various methods were employed to rid the embryos of the adhering microörganisms, but each resulted in killing the growing tips.

Seeds of the leguminous plants whose specificity of inoculation was to be tested were treated 3–4 minutes with 1:1000 solution of bichloride of mercury followed by 2–3 minutes of agitation in sterile distilled water heated to 60–64°C. They were then dried for several days and germinated in petri dishes on layers of sterile filter paper. Representative lots of the treated seeds were inoculated into nutrient broth and yeast water mannite broth to ascertain the efficiency of the treatment. Lack of microbial growth in the broths was taken to indicate an efficient treatment. In addition, seeds of *Samanea saman* (Jacq.) Merrill, *Albizzia lebbek* (L.) Benth., *Albizzia katangensis* Wildem., *Enterolobium cyclocarpum* Griseb., and *Mucuna gigantea* (Willd.) DC. were held in the open flame

after the mercuric chloride treatment until they were *slightly* charred. This method was resorted to with these seeds because natural germination in moist soil was found to require 6 months or more, and since efforts to scarify these hard, thick seed coats by ordinary means proved futile. Mortality as a result of the fire treatment was usually less than 10 per cent.

All plants were grown in sterile quartz sand contained in 1-gallon cans or in standard Mitscherlich pots. Prior to being filled with sand, the cans were painted with a non-toxic, rapid-drying, black paint to prevent rust. Several holes averaging about 4 mm. each in diameter were made in each can to afford drainage. The containers were filled with the dry quartz sand, and the tops were covered with thick wrapping paper and sterilized 8 hours on each of three successive days at 20 pounds pressure. Planting was made immediately after the third sterilization period.

The germinating seeds of the plants to be tested were placed on the moistened surface of the sand, and inoculation was made by adding several cubic centimeters of a 10-day-old broth culture of the organism in question. The seeds were then covered with sterile dry sand. The plants were watered as often as necessary with sterile distilled water and received sterile Crone's solution weekly.

Eight control pots were usually stationed at random on each table of 12 inoculated pots. Contamination was practically nil on the control series as observed by a close examination of the root systems of each plant. When the slightest contamination did occur, the data on the inoculated pots of the table or tables concerned were discarded.

Discussion and results of the plant tests

The classification of the tropical plant species into cross-inoculation groups followed two lines of approach. In the initial tests the newly isolated Rhizobia cultures from the tropical species were used as inocula for plants representative of the more common cross-inoculation groups, whereas in the later tests the reciprocal cross was attempted using as inocula Rhizobia cultures representative of the various plant groups upon seeds of the tropical species. The only exception to this two-fold procedure was the testing of the tropical seed against cultures of *Rhizobium japonicum* (Kirchner) Baldwin and Fred—an instance where lack of seed became an obstacle. However, in the counterpart of this particular series soybean and cowpea plants were grown in the same pots, both being inoculated simultaneously with the Rhizobia from the tropical species. In such experiments no occasion occurred wherein nodules were produced upon the soybean plants, whereas in every instance the root systems of the cowpea plants, intertwined with those of the soybean plants, were well nodulated.

Five stock laboratory strains of Rhizobia isolated from *Vigna sinensis* L. were used in the plant tests. Each of these strains is a beneficial inoculant, produces abundant nodules about the tap root systems of cowpea plants (*Vigna sinensis* L.), and has benefited the growth of cowpeas by the fixation of nitrogen

on repeated tests. These strains have also been used in cultures distributed for the inoculation of cowpea seed under field conditions.

Subsequent to establishing the nodule-forming abilities of the Rhizobia strains from the tropical leguminous species, five cultures were selected for use in the cross-inoculation tests, care being taken in each case to choose cultures representative of different nodules found on the plant species. A positive reciprocal cross-inoculation in the cowpea group occurred with each of the species listed in table 1, i.e., the strains of cowpea bacteria satisfactorily inoculated and enhanced the growth of the tropical plant species, and in turn the Rhizobia isolated from the nodules of the tropical leguminous species were beneficial to cowpea plants. The word "positive" may be taken to indicate a minimum of 15 nodules per plant, which was in every case accompanied by an appreciable fixation of nitrogen in comparison with the uninoculated plants.[3] Therefore, in the opinion of the authors the results obtained warrant the addition of 87 plant species to the cowpea cross-inoculation group, provided *Vigna sinensis* L. is accepted as an indicator test plant for that group. Four of the species in table 1; namely, *Crotalaria incana* L., *C. anagyroides* H. B. and K., *C. usaramoensis* Baker, and *Clitoria ternatea* L. were included in the Carroll list (3). Of the new additions reported in this paper, 72 species are trees and shrubs, and the remaining 15 species are herbs and vines.

The conclusions from these plant tests have been based upon three replicative greenhouse experiments in which each culture was run in triplicate.

Tropical species versus inoculation. All of the tropical plant species tested were inoculated by their own Rhizobia strains respectively, as well as by the cultures of cowpea bacteria. In a few exceptional cases, such as with *Samanea saman* (Jacq.) Merrill and the species of Erythrina, the host strains seemed to be more beneficial on their respective host plants as evidenced by the abundance and type of nodules produced and by the growth and color of the plants.

Characteristic differences in color of the plants and in luxuriance of growth occurred in all cases between the inoculated and the uninoculated tropical species. Such differences were more pronounced with the plants in the sub-family Papilionoideae. With the exceptions of *Mucuna gigantea* (Willd.) DC. and *Pongamia pinnata* (L.) Merrill, the seeds of the plants in this sub-family were relatively small, and therefore a stunted growth occurred as a result of nitrogen hunger in the uninoculated plants. The response to inoculation by members of this sub-family was very satisfactory. On the other hand, the seeds of the species in the Mimosoideae and Caesalpinioideae were considerably larger, and much growth occurred before nitrogen hunger appeared (in the control series). With several of the species in these sub-families, such as *Enterolobium cyclocarpum* Griseb. and *Parkia javanica* (Lam.) Merrill, it was believed that pot-binding inhibited the maximum differences in growth that would have resulted. It appeared that some of the Albizzia species were not

[3] For the sake of brevity, nitrogen analyses of the plants by the Gunning-Kjeldahl method have been omitted in this report.

suited to sand culture, since they grew very slowly and attained a height of only 8–10 inches in a 3-month period. Marked distinctions in color, however, were evident, the controls being very yellow, losing leaflets, and becoming bare, whereas the inoculated plants maintained a dark green appearance yet showed a stubby growth.

Plate 1, figure 1 shows an example of the results obtained by the inoculation of seeds of *Enterolobium cyclocarpum* Griseb. with the host strain 34-7 and with cowpea 10. The control plants were very yellow, and the inoculated plants a dark green. The finely pinnate leaf system of the species accounts for the droopy appearance of the plants. Figures 2 and 3 of plate 1 show the root system of one plant from each of the inoculated pots. Each plant of *Enterolobium cyclocarpum* Griseb. inoculated with strain 34-7 averaged 76 nodules per plant and 4.0 gm. dry weight, whereas each of the plants inoculated with cowpea strain 10 approximated 100 nodules but only 3.1 gm. dry weight. The control plants were without nodules, showed evidences of nitrogen hunger, and averaged only 1.8 gm. dry weight per plant. Figures 1, 2, and 3 of plate 2 show somewhat similar results obtained when seeds of *Erythrina indica* Lam. were inoculated, although the Erythrina cultures, as evidenced by numbers and types of nodules and by color and dry weights of the plants, were more effective on the plants of *Erythrina indica* than were cowpea cultures.

It is not within the scope of this report to deal with the various examples of host specificity and strain variation observed, since the primary purpose of this study was merely to ascertain the status of cross-inoculation. Mention should be made, however, that the cultures isolated from *Samanea saman* (Jacq.) Merrill showed an exceptional example of host specificity. All of the Samanea cultures, as well as the cowpea strains, beneficially inoculated plants of *Samanea saman* (Jacq.) Merrill as evidenced by the color and growth and by the type of nodules on the Samanea roots, whereas, the cowpea plants when inoculated with the Samanea cultures produced only small well-scattered nodules, and only slight differences in growth occurred between the control and treated plants. By analysis the cowpea plants inoculated with the *Samanea saman* cultures averaged 2.9 per cent nitrogen in comparison with an average of only 1.21 per cent nitrogen in the control cowpea series. Additional tests showed the same general type of results when the Samanea cultures were used to inoculate seeds of *Acacia koa* A. Gray, *Cajanus cajen* (L.) Millsp., and *Crotalaria juncea* L. Figure 4, plate 2, shows the type of nodule produced on pigeon pea plants [*Cajanus cajan* (L.) Millsp.] by cowpea strain 10, and figure 5, plate 2, illustrates the type of nodule production by the strains of *Samanea saman* (Jacq.) Merrill.

Although Rhizobia were not isolated from nodules of the following species, it seems quite logical to assume that these species belong to the cowpea group, since plants of each were inoculated with cowpea nodule-forming bacteria. It might be fitting, however, that definite inclusions of these species within the cowpea group await data pertaining to the inoculation of cowpea plants with

these specific Rhizobia strains. In this regard, Rhizobia cultures of *Acacia koa* A. Gray, strains 15-3 and 15-4, and cowpea strains 10 and 12 satisfactorily inoculated seeds of *Acacia koa* var. *lanaiensis* Rock, *Acacia koa* var. *hawaiiensis* Rock, and *Acacia kauaiensis* Hillbr. Likewise cowpea cultures 10 and 12 and strains 9-2 and 9-4 of *Crotalaria sericea* Retz satisfactorily inoculated seeds of the following species of Crotalaria obtained from various experiment stations:

C. cunninghamii R. Brown
C. sphaerocarpa Perrott
C. natalitia Meisn.
C. astragalina Hochst.
C. fulva Roxb.
C. longirostrata Hook. and Arn.
C. tetragona Roxb.
C. grantiana Harvey

Present status of the cowpea group. At the present time two detailed summarizations of the cowpea group appear in the literature, one by Walker (19) in 1928, the other by Fred, Baldwin, and McCoy (4) in 1932. It becomes apparent from a comparison of the two summarizations that the latter compilation is the more accurate; thus a total of 21 genera and 41 species constituted the nucleus to which Carroll in 1934 (3) added 30 species comprising 8 genera. Five of the latter genera, Apios, Aeschynomene, Erythrina, Mimosa, and Clitoria were new to cross-inoculation grouping.

The data presented in this report warrant, in the opinion of the authors, the addition of 30 species of 16 new genera and 57 species of 10 previously placed genera to the cowpea group. These results were obtained by the inoculation of seeds of the tropical species with cowpea nodule bacteria and the reciprocal inoculation of cowpea seed by the newly isolated Rhizobia from these tropical species. Three exceptions to this procedure have been noted in the preceding discussion, i.e., species of Inga, Hymenaea, and Andira.

Since the establishment of the cowpea group, additional experimental evidence has shown it to consist of a versatile and cosmopolitan collection of leguminous plants. Burrill and Hansen (2) in 1917 were probably the first to note this dissimilarity to the other cross-inoculation groups. In the past 18 years the addition of various herbs, vines, shrubs, and trees have served to make the versatility of the group more obvious.

It is generally accepted that a cross-inoculation group implies a group of plants in which the nodule-forming bacteria are mutually interchangeable. Whether the now so-called "cowpea group" complies with this definition might well be worthy of experimental consideration. In practically all of the evidence heretofore offered, only *Vigna sinensis* L. and the nodule bacteria therefrom have been used as indexes for the addition of species into the group, whereas the smaller number of species in the other groups have permitted a greater range of host tests.

Recently Carroll (3) has shown some interesting data concerning irregularities in nodule formation by cowpea bacteria on supposedly interchangeable host plants within the cowpea group. He reported several cases wherein host plants accepted as *bona fide* members of the cowpea group failed to produce nodules when inoculated with Rhizobia from other plants in the same group. In practically all instances a greater number of nodules occurred on the roots of *Vigna sinensis* L. than on the other host plants regardless of the source of inoculum. Such irregularities are so far without explanation.

Various investigators have emphasized the close relationship existing between the root nodule bacteria of the cowpea and soybean groups. As early as 1917 Burrill and Hansen noted the similarity in growth characteristics of the bacteria from the two groups. Others have confirmed these similarities. In 1921 Löhnis and Hansen (10) and Shunk (17) showed that the cowpea and soybean bacteria were similar in that they possessed monotrichous flagella, whereas the bacteria from the other groups were peritrichously flagellated. Similarity in reactions has also been noted in litmus milk (10).

According to Sears and Carroll (16), C. D. Jones was the first to show a kinship in nodule-forming ability between the two groups: his results showed that a culture of soybean nodule bacteria produced nodules upon cowpea as well as upon soybean roots. Since then Leonard (7), Sears and Carroll (16), Hansen and Tanner (6), Walker and Brown (20), and Carroll (3) have cited additional evidence showing that such interchangeability in nodule-forming function does occur. This interchangeability, however, has not been perfect in all cases. In the majority of instances soybean cultures have possessed the power to induce nodules on the roots of cowpea, whereas only a few of the cowpea cultures tested have infected the soybean plant. Recently Walker and Brown (20) have proposed that the root nodule bacteria of the cowpea and soybean groups be considered as the one species now generally recognized as *Rhizobium japonicum* (Kirchner) Baldwin and Fred.

Serological tests have given enlightening evidence on the cross-inoculation status of various plants but have not been free of incongruous results. The recent contribution of Hansen and Tanner (6) has shown that the correlation between cross-inoculation and cross-agglutination is not so direct as might be inferred from the work of Simon (18). Yet a relation has been shown to exist between serological behavior and the cross-inoculation phenomenon, despite the fact that it is not a reciprocal one. Whether serological methods may become basic in cross-inoculation controversies seems questionable. Recently results have shown that all strains from a single cross-inoculation group did not cross-agglutinate. Hansen and Tanner (6) have recently shown six cultures from the cowpea group to fall into three agglutination groups, five cultures of which were isolated from cowpea plants. Seven soybean cultures were divided into two agglutination groups. In addition two strains of cowpea bacteria cross-agglutinated with two strains of the soybean organism. Thus, more than

one serological strain has been isolated from the same host, yet no two serologically similar nodule-forming bacteria from different host plants have been found which failed to cross-inoculate.

Inasmuch as the criterion for species differentiation within the genus Rhizobium has been the ability to inoculate plants, the question of host affinities becomes of greater significance. It seems questionable that the evidence to date warrants the consolidation of the soybean and cowpea groups under one species of Rhizobium. In some cases the numbers of nodules produced on plants in the cross-inoculation tests have been too few to warrant the importance attributed to them. In other cases plus signs have been used to indicate the extent of nodulation, but explanatory remarks have not elucidated whether the plus signs signified one nodule or two dozen nodules. Although it is admitted that too much significance cannot be placed upon the actual numbers of nodules produced on a plant by a given culture, yet, in instances of this nature, numbers are of more value than symbols. Whether the soybean bacteria benefit cowpea plants and *vice versa* may be a technicality aside from the main issue of the ability of the bacteria to cross-inoculate, but it is decidedly pertinent from an agricultural viewpoint. Data of this nature have not been presented. Moreover, the small number of soybean and cowpea cultures and plants tested to date by the various authors should not be taken to represent the far greater number of plants involved. A greater variety and number of host plants and Rhizobia, along the line previously presented by Carroll (3), need to be tested. Before the consolidation of the two groups becomes generally recognized, interchangeability of cowpea and soybean bacteria on the host plants of the two groups should affect a normal occurrence and not exceptions to the general rule.

Whether the consolidation of the soybean and cowpea groups is a logical move at this time is doubtful. It is very likely that the cowpea group, now so very large and unwieldy as a unit in the sense of the present definition of a cross-inoculation group, could better be subdivided into smaller or sub-groups based on susceptibility to certain strains of Rhizobia from plants more or less closely related, and yet maintain some degree of unity. It is very probable that the soybean plant would bear a close relationship to certain ones of these groups.

SUMMARY

The data in this report have dealt with observations on nodule formation of various tropical leguminous plants and cross-inoculation tests with these species.

The versatility of the now so-called "cowpea group" has been further emphasized by the addition of various distantly related leguminous plants as a result of rigidly controlled plant inoculation tests.

The present status of the cowpea group is briefly discussed.

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PLATE 1

FIG. 1. Response of *Enterolobium cyclocarpum* to inoculation.

Left, control, no inoculation. Center, inoculated with strain 34-7, isolated from a nodule of *Enterolobium cyclocarpum*. Right, inoculated with strain 10, isolated from *Vigna sinensis*. Age of plants, 3½ months.

FIG. 2. Nodule formation on *Enterolobium cyclocarpum* inoculated with strain 34-7.

FIG. 3. Nodule formation on *Enterolobium cyclocarpum* inoculated with cowpea strain 10.

FIG. 4. Nodules from *Acacia horrida* growing in forest soil. One-half natural size.

FIG. 5. Nodules from *Inga edulis* growing in field soil. One-half natural size.

FIG. 6. Nodules from *Andira inermis* growing in forest soil. One-half natural size.

FIG. 1

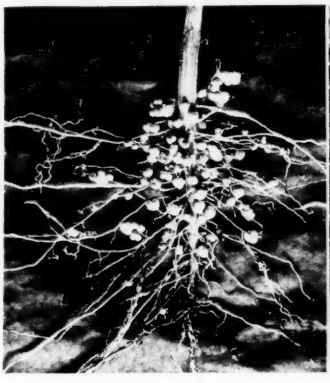

FIG. 2

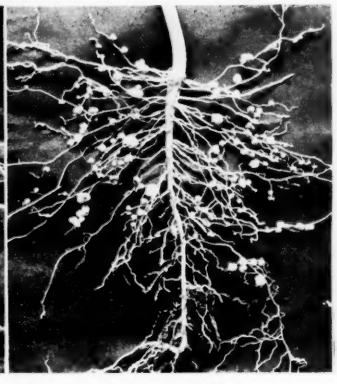

FIG. 3

FIG. 4

FIG. 5

FIG. 6

PLATE 2

FIG. 1. Response of *Erythrina indica* to inoculation.

Left, control, no inoculation. Center, inoculated with strain 36-4, isolated from a nodule of *Erythrina indica*. Right, inoculated with strain 10 isolated from *Vigna sinensis*. Age of plants, 3 months.

FIG. 2. Nodule formation on *Erythrina indica* inoculated with strain 36-4.

FIG. 3. Nodule formation on *Erythrina indica* inoculated with cowpea strain 10.

FIG. 4. Nodule formation on the roots of *Cajanus cajan* inoculated with cowpea strain 10.

FIG. 5. Nodule formation on the roots of *Cajanus cajan* inoculated with strain 13-5, isolated from a nodule on *Samanea saman*.

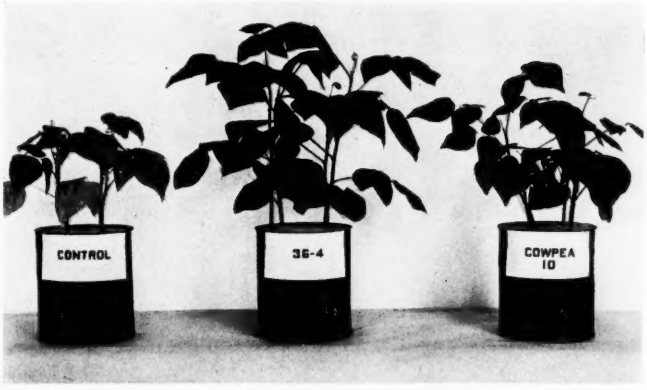

FIG. 1

FIG. 2

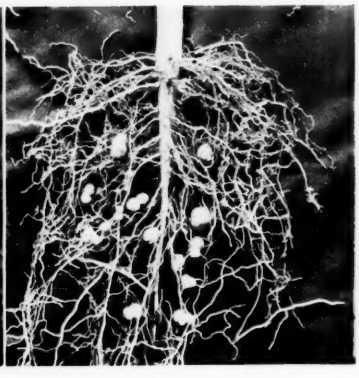

FIG. 3

FIG. 4

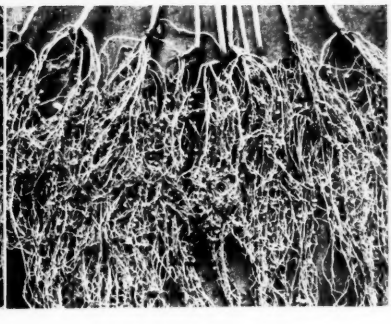

FIG. 5